BASOLATERAL AMYGDALA REGULATION OF CELLULAR AND CHEMICAL SIGNALS WITHIN THE NUCLEUS ACCUMBENS DURING CONDITIONED REWARD-SEEKING

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ABSTRACT

JOSHUA L. JONES: Basolateral amygdala regulation of cellular and chemical signals within the nucleus accumbens during conditioned reward-seeking (Under the direction of Regina M. Carelli)

Rewards are not procured and consumed in a vacuum. Rather, successful pursuit of reward occurs in a diverse, dynamic environment. As such, the ability to form and maintain associations between environmental cues, actions, and rewarding stimuli is a fundamental aspect of goal-directed behaviors. The adaptive significance of these associative processes is abundantly clear, as organisms that can better predict, procure and consume rewards using environmental cues will enjoy increased success in future decision-making. Numerous lines of research have identified that associative reward processing is mediated by a distributed network of brain nuclei that includes the nucleus accumbens (NAc) and its innervation from dopamine neurons located in the midbrain. However, the precise neural processing, and neural circuitry that mediates these associative processes remain unclear. This dissertation seeks to dissect the contribution of one corticolimbic input, the basolateral amygdala (BLA), to neurochemical and neurophysiological signaling within the NAc. The first set of experiments detailed in this dissertation took advantage of technological advances to characterize patterns of NAc dopamine release in real time, during cued-instrumental responding for a natural reinforcer, and determine the contribution of the BLA to these signals. The results of the first experiment demonstrate for the first time that rapid dopamine release in the NAc and
conditioned responding to reward-predictive stimuli are functionally mediated by the BLA. The second set of experiments examined the contribution of the BLA to the post-synaptic signaling of NAc neurons, demonstrating that the BLA facilitates excitatory encoding of reward-predictive cues within the NAc, during cued-instrumental responding for a natural reward. The final experiments described in this dissertation examined how these associative reward signals within the NAc and demonstrate that the BLA contributes to conditioned neural excitations within the NAc during cocaine self-administration, while also regulating the tonic firing rate of phasic inhibitions. Together, these experiments provide novel characterizations of the neural circuits and mechanisms by which environmental stimuli are processed within the brain, providing insight into the potential role of the BLA-NAc circuit in mediating psychiatric disorders, such as drug addiction, which are characterized by maladaptive goal-directed behavior and associative reward processes.
To my parents
ACKNOWLEDGEMENTS

The work presented here, while a reflection of my personal efforts, is more accurately reflects the good fortune and opportunities that I have been afforded. I would first like to express many thanks to my advisor, Dr. Regina Carelli, for the opportunity to pursue these endeavors in a rich intellectual atmosphere. Without her guidance none of this work would have been possible. I would also like to thank Dr. R. Mark Wightman for bridging the gaps between chemistry and neuroscience, and for his support and helpful discussion throughout this process. The conceptualization, design, and execution of the experiments that make up this dissertation were the result of numerous discussions, for which I would like to thank Dr. Mitchell F. Roitman, Dr. Robert A. Wheeler, Dr. Brandon J. Aragona, Dr. Jeremy J. Day, and Dr. Michael Saddoris. I would also like to acknowledge Dr. Robert A. Wheeler, Dr. Brandon J. Aragona, Dr. Jeremy J. Day, Jennifer Slater, Mark Stuntz, Kate Fuhrmann, Jessica Briley, and Laura Ciompi for their technical assistance in these experiments. Finally, I would like to acknowledge the tremendous support I have received from my wife, Jennifer Slater, my parents and family, and my friends throughout this journey. Your patience, encouragement and guidance have made this road much easier to travel. Also, many thanks to the National Institute of Health, specifically the National Institute on Drug Abuse for years of funding.
PREFACE

This dissertation was prepared in accordance with guidelines set forth by the University of North Carolina Graduate School. This dissertation consists of a general introduction, three chapters of original data, and a general discussion chapter. Each original data chapter includes a unique abstract, introduction, results, and discussion section. A complete list of the literature cited throughout the dissertation is included at the end. References are listed in alphabetical order and follow the format of The Journal of Neuroscience.
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<td>Analysis of variance</td>
</tr>
<tr>
<td>BM</td>
<td>Baclofen/muscimol treatment</td>
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<td>BLA</td>
<td>Basolateral amygdala</td>
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<td>CeN</td>
<td>Central nucleus of the amygdala</td>
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<td>CR</td>
<td>Conditioned response</td>
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<td>CS</td>
<td>Conditioned stimulus</td>
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<td>US</td>
<td>Unconditioned stimulus</td>
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<tr>
<td>VEH</td>
<td>Vehicle treatment</td>
</tr>
<tr>
<td>VP</td>
<td>Ventral pallidum</td>
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<tr>
<td>VTA</td>
<td>Ventral tegmental area</td>
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Rewards are not procured and consumed in a vacuum. Rather, successful pursuit of reward occurs in a diverse, dynamic environment. As such, the ability to form and maintain associations between beneficial actions and the environmental cues that predict them is a fundamental aspect of goal-directed behaviors. The adaptive significance of these associative processes is abundantly clear, as organisms that can better predict, procure and consume rewards using environmental cues will enjoy increased success in future decision-making. Conversely, disruption of these adaptive processes will lead to abnormal and detrimental behaviors at significant cost to the organism. As such, it becomes apparent that understanding the neural circuitry that mediate these behavioral processes may greatly inform dysfunction in human conditions, such as compulsive disease states like addiction.

However, it is also apparent that adaptive responding and goal-directed action likely includes a diverse network of brain nuclei, each contributing unique features to behavioral output. Thus, the following dissertation seeks to thoroughly examine a portion of this network, focusing on an intensely examined region, the nucleus accumbens (NAc). The NAc receives robust neural projections from various limbic structures, including the amygdala, hippocampus and prefrontal cortex (PFC) (Brog et al., 1993; Heimer et al., 1997; Zahm, 1999), in addition to robust dopaminergic innervation from the ventral tegmental area (VTA) (Phillipson, 1979; Swanson, 1982). In turn, the NAc projects to structures related to the
control of motor output, implicating a functional role as a site of convergence and integration between limbic and motor systems (Heimer et al., 1991; Zahm and Heimer, 1993). Behavioral studies have substantiated early anatomical assertions as NAc manipulations disrupt locomotor behaviors, reward processes, reinforcement, learning, and decision making (Di Chiara and Imperato, 1988; Schultz et al., 1997; Berridge and Robinson, 1998; Salamone and Correa, 2002; Wise, 2004; Frank and Claus, 2006; Nicola, 2007; Phillips et al., 2007; Humphries and Prescott, 2009). While tremendous gains have been made in characterizing how NAc signals track different reward processes, the functional contribution of these NAc signals, and the afferent control of them have not been extensively studied.

The experiments described in this dissertation seek to dissect the contribution of one corticof limbic input, the basolateral amygdala (BLA), to neurochemical and neurophysiological signaling within the NAc. Critically, the following experiments will examine the in vivo contribution of BLA activity to NAc signaling during cued-instrumental responding. Therefore, this chapter will provide a brief introduction of the exhaustive literature on the role of the NAc signaling in reward processes and goal-directed behavior. This chapter will first review basic concepts related to associative reward processes and goal-directed action. Secondly, this chapter will discuss the neural substrates that mediate these reward processes, with emphasis on the NAc and its dopaminergic input from the VTA, and the corticof limbic input from the BLA. Thirdly, this chapter will review the cellular and systems-level mechanisms underlying neural communication within the NAc. Finally, these ideas will be integrated in order to examine theoretical and empirical links between BLA neural activity and NAc dopamine release and neural activity, specifically mediating conditioned reward-seeking behaviors.
**Associative reward processing and goal-directed action**

Organisms are constantly pursuing critical goals in highly dynamic and demanding environments. Evolution has imbued organisms with the ability to associate important environmental stimuli with successful reward-seeking and goal-directed actions, allowing adaptive behavioral strategies to develop through application of predictive information. The motivational control of goal-directed actions is generally thought to occur through two well-described associative processes. The first, commonly referred to as Pavlovian conditioning, occurs as organisms associate a previously neutral stimulus (the conditioned stimulus, or CS) that is paired with a biologically salient event such as the delivery of food (the unconditioned stimulus, or US), which elicits an unconditioned response (UR). These CSs can influence ongoing behavior by generating both preparatory and consummatory conditioned responses (CR) (Pavlov, 1927; Konorski, 1967; Brown and Jenkins, 1968; Jenkins and Moore, 1973). It is possible then for these Pavlovian associations to different types of US representations within the brain. For example, the CS can represent the simple stimulus-response association, or represent the affect associated with the CS, or the CS may represent specific sensory properties of the the US itself (Dickinson and Mackintosh, 1978; Everitt et al., 2000; Cardinal et al., 2002). These associative processes are sensitive to a number of factors, including the temporal delay between the CS and US, the frequency of CS-US pairings, the intensity of stimuli employed, and the contingency between the CS and the US, or the degree to which the CS predicts the US (Rescorla, 1968, 1969, 1988).

The second, termed instrumental conditioning, is largely mediated through the contingency between actions or responses with biologically salient outcomes, and thus those actions increase or decrease in frequency (Thorndike, 1933; Skinner, 1938, 1981). Akin to
Pavlovian processes, the frequency of responding observed following instrumental conditioning is subject to a number of variables, including the rate of reinforcement, the number of responses required for reinforcement, and the concurrent presence of other reinforcers. Over time, such instrumental responses can become habitual, and are more dependent upon the stimuli that precede them than the outcome that follows them (Watson, 1913; Dickinson, 1994). In contrast, goal-directed instrumental responses are identified by two critical criteria: 1) sensitivity to the contingency between the action and the outcome and 2) sensitivity to changes in the value of the outcome, such as devaluations (Balleine and Dickinson, 1992; Dickinson et al., 1996; Balleine and Dickinson, 1998).

While these two associative processes are often described separately, in reality they are intricately connected, as highly complex interactions between them are constantly present as organisms perform even the simplest behaviors (Rescorla and Solomon, 1967). For example, under instrumental contexts, environmental cues play an important role in guiding appropriate action. Specifically, environmental cues that predict rewards contingent upon a response (here called discriminative stimuli; DS) play a critical role in signaling if and when behavior will be reinforced and guide subsequent actions accordingly. Although these DS have clear Pavlovian characteristics, they have effects that cannot be readily explained through simple CS-US associations (Rescorla, 1990, 1994). Furthermore, CSs can serve as instrumental reinforcers (Zimmerman, 1957), demonstrating that they also maintain their own reinforcing and incentive properties. Moreover, the presentation of Pavlovian cues can exert robust motivational effects on instrumental actions, even when there is no specific connection between the cue and the response. In this phenomenon, known as Pavlovian-to-instrumental transfer (PIT), animals that were separately trained to associate a CS with
delivery of a US and to press a lever for delivery of the same US are then presented with the CS in the instrumental context under extinction. Under this condition, presentation of the Pavlovian CS increases response rates, demonstrating its ability to augment instrumental responding (Estes, 1948; Holland, 2004).

As they relate to rewarding or reinforcing stimuli such as food, water, and copulation, these associative processes are fundamental and clearly adaptive in that animals are better able to predict, procure and consume future rewards and goals. However, natural environments present organisms with a complex array of response options that compete for behavioral resources (Stevens and Krebs, 1986). Therefore, once organisms have learned the predictive relationship between stimuli and rewards or actions and rewards, they must use this information to guide and optimize future behavior. It becomes evident that elucidating and dissociating the underlying neural circuitry of these associations will greatly inform our understanding of goal-directed action and reward processing.

**The nucleus accumbens**

Decades of research have identified a diverse network of brain nuclei involved in numerous aspects of reward processing. The NAc is one of the most extensively studied neural substrates within this network, particularly with regard to reward processing. The NAc is defined as the region bounded rostrally and laterally by the external capsule, and medially by the septum and lateral ventricle (Voorn et al., 1989; Groenewegen et al., 1991; Zahm and Brog, 1992). Caudally, the NAc merges with the bed nucleus of the stria terminalis, while ventrally it merges with the olfactory tubercle (Voorn et al., 1989; Groenewegen et al., 1991). The dorsal border with the remainder of the striatum, is only
delineated using a variety of histochemical criterion (Zaborszky et al., 1985; Voorn et al., 1989; Groenewegen et al., 1991).

**NAc cellular and chemical composition**

The neuronal composition of the NAc is predominantly comprised of medium spiny neurons (MSNs), which form greater than 90% of the neuron population within the rat (Groves, 1983; Gerfen and Wilson, 1996). MSNs are GABAergic neurons and are the primary output neurons of the NAc. MSNs are noted for their closed-field morphology, with numerous dendritic radiations emanating from the soma, and possess a thin, unmyelinated axon that gives off local collaterals which contact the dendritic branches of neighboring MSNs (Wilson and Groves, 1980; Groves, 1983; Kawaguchi, 1993). Immunohistomchemical markers reveal that MSNs contain enkephalin, dynorphin, substance P, and neurotensin (Voorn et al., 1989). Additionally, as a primary terminal region for dopamine neurons, MSNs express at least one class of dopamine receptor (D1-like or D2-like), with enkephalin-containing MSNs exhibiting higher levels of D2 receptor expression, whereas dynorphin positive neurons exhibit greater D1 receptor expression (Le Moine and Bloch, 1995). Importantly, the neurophysiological properties of MSNs are rather unique. In slice preparations, MSNs exhibit a bistable membrane potential which is characterized by a hyperpolarized “down-state” resting at ~ -85mV, and a depolarized “up state” which approaches the threshold for spike generation, ~ -60mV (Wilson and Kawaguchi, 1996). Moreover, the transition between these states is mediated by synaptic inputs, and MSNs are only able to generate action potentials while in the “up state” (Nicola et al., 2000; O'Donnell, 2003).
The remaining 5-10% of neurons within the NAc is comprised of at least three main interneuron classes. The first is the giant aspiny cholinergic interneuron (Groves, 1983), which is characterized by a relatively large soma, radially emanating dendrites, and short myelinated axons (Kawaguchi, 1993; Kawaguchi et al., 1995). Recent neurophysiological evidence suggests these neurons are characterized by long-lasting afterhyperpolarizations, and correlate with the tonically active neurons seen in vivo (Kawaguchi et al., 1995; Cragg, 2006). The second group is the aspiny medium GABAergic interneuron (Kawaguchi, 1993). These neurons co-express parvalbumin and appear to be correlated with the physiological class of fast-spiking interneurons (Kawaguchi et al., 1995). The third group contains the interneurons that co-express nitric oxide, somatostatin and neuropeptide Y (Tepper and Bolam, 2004). This subpopulation likely releases GABA, and correlate with the physiologically low-threshold spiking class of neurons that have been characterized (Kawaguchi et al., 1995; Kubota and Kawaguchi, 2000). Evidence suggests that these subpopulations of interneurons are functionally distinct from one another (Berke et al., 2004; Berke, 2008; Berke et al., 2009).

Anatomic subregions: the core and shell

The NAc is a heterogenous structure, comprised of two primary subregions that can be dissociated both anatomically and functionally. The core subregion is typically defined as the densely packed cells surrounding the anterior commissure, which is then bordered medially and ventrally by the shell subregion (Zahm and Brog, 1992). These subregions can be identified anatomically through histochemical staining for different enzymes and proteins, including acetylcholinesterase (ACHe) and calbindin (calcium-binding protein), both of which provide the primary methods for dissociating the borders between the core and shell.
(Zaborszky et al., 1985; Voorn et al., 1989; Groenewegen et al., 1999). Furthermore, the core and shell exhibit differential neurotransmitter binding, including differences in dopamine, GABA, and opioid receptor binding. Expression of the dopamine transporter is also different across subregion (Nirenberg et al., 1997). While both core and shell are primarily composed of MSNs, these neurons exhibit different morphological characteristics, in that shell neurons are smaller, with fewer dendrites and a lower spine density (Meredith et al., 1992). These morphological differences presumably underlie the differences observed in the membrane properties of these MSNs (O'Donnell and Grace, 1993).

**Afferent and efferent projections.**

The rodent NAc receives afferent projections from a variety of cortical and subcortical structures, including the basolateral amygdala (Kelley et al., 1982; McDonald, 1991; Zahm and Brog, 1992; Brog et al., 1993; Wright et al., 1996), the prefrontal cortex (McGeorge and Faull, 1989; Zahm and Brog, 1992; Brog et al., 1993), the subiculum of the hippocampus (Groenewegen et al., 1987; Groenewegen et al., 1991; Zahm and Brog, 1992; Brog et al., 1993), and a dense dopaminergic projection from the ventral tegmental area (Zahm and Brog, 1992). NAc neurons in turn impact behavior through their projections to the substantia nigra, ventral pallidum, and lateral hypothalamus (Zahm, 1999). However, the afferent and efferent projections within the NAc are not homogenously distributed across the core and shell. For example, the primary cortical afferents to the shell and core originate in differing cortical subregions (i.e. the orbitofrontal, infralimbic and posterior piriform to the shell; the dorsal prelimalbic and anterior cingulated to the core) (Vertes, 2004). Additionally, several other afferent inputs are topographically organized (medial VTA projects to the medial shell and more lateral regions of the VTA project to the core and lateral shell).
With respect to efferent projections, the shell innervates the ventromedial portion of the ventral pallidum (VP), the lateral pre-optic lateral hypothalamus, and the VTA (Zahm and Heimer, 1993). The core rather tends to project to the dorsolateral VP, the subthalamic nucleus, and more lateral regions of the VTA and portions of the substantia nigra reticulata (Zahm and Heimer, 1993).

Given the anatomic arrangement of the NAc (Figure 1.1), it was proposed by Mogenson (Mogenson, 1987) and elaborated upon by others (Everitt and Robbins, 1992; Pennartz et al., 1994; Ikemoto and Panksepp, 1999) that the NAc functions as a site for the integration of limbic information related to memory, motivation and generation of flexible behaviors necessary for adaptive motor responding. Subsequent observations have demonstrated that NAc afferents make convergent synaptic contacts on to MSNs. For example, immunocytochemistry and electron microscopy have revealed that inputs from the BLA are apposed to dopaminergic inputs from the VTA (Totterdell and Smith, 1989; Sesack and Pickel, 1990). Likewise, Van Bockstaele and Pickel (Van Bockstaele and Pickel, 1993) reported that 5-HT terminals were in direct contact with dopaminergic axons. In addition, a convergence of inputs from the medial prefrontal cortex and the ventral subiculum on NAc neurons has been identified (French and Totterdell, 2002) as well as the BLA and ventral subiculum (French and Totterdell, 2003). Taken together, these findings suggest that NAc neural activity is an integration of afferent information and indicate that multiple NAc afferents are capable of influencing NAc cell firing in behaving animals (Pennartz et al., 1994; O'Donnell and Grace, 1995; Carr and Sesack, 2000; Pinto and Sesack, 2000).

*Synaptic actions within the NAc*
NAc neurons express several forms of glutamate receptors, including AMPA, NMDA, kainite and several forms of metabotropic glutamate receptors (Gracy and Pickel, 1996; Lu et al., 1999). Most of these receptors are located within the synapses located on the spines of the MSN dendrites, where glutamatergic terminals form asymmetrical synapses (Sesack and Pickel, 1990). Additionally, dopamine axons terminate onto the necks of synapses in MSNs, mostly at locations where the head of the striatal synapse also receives an excitatory input (Groves et al., 1994; Moss and Bolam, 2008). This anatomical arrangement, together with a wealth of *in vitro* studies, suggests that instead of having direct excitatory or inhibitory actions, dopamine serves to modulate ongoing activity at glutamatergic synapses (O'Donnell et al., 1999; Nicola et al., 2000; Brady and O'Donnell, 2004; Goto and Grace, 2005). As mentioned above, MSNs exhibit bistable membrane potentials that are driven by convergent synaptic input (Nicola et al., 2000). *In vivo*, one effect of dopamine may be to “gate” glutamatergic inputs in the NAc, such that only the strongest inputs can control NAc output (Nicola et al., 2000; Floresco et al., 2001b; Floresco et al., 2001a). Glutamatergic synapses at MSNs undergo bidirectional synaptic plasticity (long term potentiation, LTP; long-term depression, LTD) as a result of patterned stimulated activity or administration of certain drugs (Kombian and Malenka, 1994; Nicola et al., 2000; Thomas et al., 2001). Recent evidence suggests that dopamine may direct this synaptic plasticity, in effect determining which synapses become augmented or attenuated by activity (Thomas et al., 2000; Boudreau et al., 2007; Kourrich et al., 2007; Conrad et al., 2008; Shen et al., 2008). Dopamine receptor activation is required for the induction of synaptic plasticity at MSNs, and the overall effect of dopamine is dependent upon the type of dopamine receptor expressed within the MSN (Pawlak and Kerr, 2008; Shen et al., 2008). This arrangement suggests a mechanism by
which temporally coincident stimulation of dopamine and glutamate receptors can initiate complex intracellular cascades that mediate changes in gene expression in a specific set of neurons (Kelley, 2004b; Valjent et al., 2005; Stipanovich et al., 2008).

Figure 1.1. Simplified schematic of afferent and efferent connections of the NAc. Note, these are not indicative of precise anatomical location or degree of projections. Blue arrows denote GABA; Green arrows denote Glutamate; Red arrows denote Dopamine.

The NAc and reward

The contribution of the NAc to reward and motivation has heavily emphasized the role of NAc dopamine (Berridge and Robinson, 1998; Berke and Hyman, 2000; Wise, 2004). However, while the importance of dopamine to motivated behavior is critical, dopamine itself does not directly drive post-synaptic signaling of MSNs. As such, it is important to consider the contributions of NAc processing as a whole in an attempt to better understand the neural circuitry of reward processes.

Early studies demonstrated that the NAc contributes to both the appetitive and consummatory phases of reward responding (Stratford and Kelley, 1997; Swanson et al.,
Both GABA agonism and glutamate antagonism in the NAc produce increases in food consumption, indicating that neuronal inhibition of the NAc may play an important role in the initiation or maintenance of feeding behavior (Kelley, 2004b). Intra-NAc μ-opioid agonists have also been shown to augment food intake, while animals receiving μ-opioid antagonists exhibit attenuated consumption (Kelley et al., 1996; Pecina and Berridge, 2000). Interestingly, manipulations that increase food intake are most effective in the shell of the NAc, further indication of subregion functional dissociations. In addition, a spatially restricted area within the medial NAc shell has been specifically implicated in the ability of opioid agonists to alter hedonic reactions to both rewarding and aversive stimuli (Pecina and Berridge, 2005). Thus, some categories of reward-related information may be processed by distinct neurotransmitter systems in functionally isolated regions of the NAc.

The functional role of the NAc and its dopaminergic innervation during Pavlovian conditioning has been explored extensively using site-specific lesions and pharmacological manipulations. Importantly, functional dissociations between NAc core and shell subregions have been identified. For example, in an autoshaping paradigm in which rats are trained to associate the presence of a previously neutral stimulus with the delivery of a food reward, selective lesions were made to either the core or shell of the NAc. Rats underwent additional pairing sessions in which conditioned approach responses towards the reward-paired cue were monitored (Parkinson et al., 1999). Lesions to the NAc core (but not shell) significantly impaired the expression of these approach responses, indicating that CS-US associations were disrupted. Similarly, dopamine antagonism or depletion in the NAc core also produces a profound impairment in the ability of animals to learn and express conditioned approach...
responses (Di Ciano et al., 2001; Parkinson et al., 2002). By comparison, NMDA antagonism in the NAc disrupts conditioned responses only during acquisition, whereas AMPA antagonism preferentially impairs the expression of Pavlovian approaches (Di Ciano et al., 2001). Taken together, these findings suggest that the NAc core is critical for the maintenance of conditioned approach responses.

Interestingly, the NAc does not seem to regulate the ability to respond for a CS acting as a reinforcer (conditioned reinforcement) (Taylor and Robbins, 1986; Parkinson et al., 1999). This is of great import, as it suggests that although the NAc clearly has a role in mediating certain Pavlovian influences over behavior, it is not necessary for the Pavlovian association to be formed (Cardinal et al., 2002). Furthermore, while lesions may reveal the necessity of a structure, these findings do not eliminate the ability to greatly modulate these behaviors. For example, psychostimulant drugs have the ability to significantly enhance responding for conditioned or secondary reinforcement (Taylor and Robbins, 1984; Cador et al., 1991; Wolterink et al., 1993). Numerous studies have demonstrated that the integrity of the NAc shell and its dopamine innervation greatly mediate these effects (Cador et al., 1991; Wyvell and Berridge, 2000; Ito et al., 2004). Furthermore, more recent studies have demonstrated that the core and shell can be dissociated in their contribution to conditioned reinforcement of a drug-paired CS, as transient inactivation of the NAc core, but not the shell, inhibits expression of this response (Di Ciano et al., 2008).

While conditioned reinforcement may or may not be critically dependent upon the NAc, there is extensive evidence to suggest that the ability of a Pavlovian CS to invigorate responding for a separately trained instrumental response is dependent on NAc function (Corbit et al., 2001b; Hall et al., 2001; Murschall and Hauber, 2005; Lex and Hauber, 2008).
Specifically, the PIT effect is eliminated by lesions of the NAc, and systemic or intra-NAc dopamine receptor manipulations (Hall et al., 2001; Lex and Hauber, 2008). Moreover, lever pressing in the presence of Pavlovian cues is robustly enhanced by intra-NAc administration of amphetamine, which increases dopamine transmission (Wyvell and Berridge, 2000).

Although dopamine and NAc activity appear to have a clear role in Pavlovian learning, their specific role in instrumental responding remains somewhat controversial (Kelley, 2004b; Fields et al., 2007; Belin et al., 2008; Yin et al., 2008b). For example, studies have found that NMDA and D1 receptor antagonism in the NAc disrupts learning in instrumental tasks (Maldonado-Irizarry and Kelley, 1995; Kelley et al., 1997; Smith-Roe and Kelley, 2000). Moreover, inhibition of the downstream signaling cascades and alterations in protein expression, such as protein kinase A or protein synthesis in the NAc also disrupts this instrumental responding (Baldwin et al., 2002; Hernandez et al., 2002). Conversely, other studies have found that neither dopamine nor an intact NAc are required for instrumental learning (Corbit et al., 2001a; de Borchgrave et al., 2002; Cardinal and Cheung, 2005; Robinson et al., 2005) or instrumental responding per se (McCullough et al., 1993; Balleine and Killcross, 1994; Aberman and Salamone, 1999). It should be noted though, that these studies may not always dissociate performance deficits adequately, which may underlie some inconsistencies. However, it is clear that an intact NAc is not necessary for goal-directed instrumental responding, which also leads to a great deal of confusion (Yin et al., 2008b). As such, it is likely the case that the NAc is critical for aspects of motivation that promote responding for rewards in response to environmental stimuli (Everitt et al., 2000; Cardinal et al., 2002; Yin et al., 2008b), as there is widespread agreement that NAc manipulations can dramatically alter behavioral performance in instrumental tasks that are mediated by
environmental cues (Ikemoto and Panksepp, 1999; Balleine, 2005; Fields et al., 2007; Nicola, 2007; Yin et al., 2008b). For example, dopamine antagonism in the NAc or VTA inactivation reduces lever presses evoked by reward-paired discriminative stimuli (Yun et al., 2004a; Yun et al., 2004b), and manipulations that increase NAc dopamine also increases the number of cues to which animals respond (Nicola et al., 2005). Furthermore, conditioned place preference (Tzschentke, 1998), and cue-induced reinstatement (Fuchs et al., 2004), two paradigms that integrate Pavlovian influences on instrumental action, are attenuated through disruption of NAc processing.

It is clear that the NAc is a key neural substrate mediating aspects of reward processing. The structure mediates the ability of CSs to invigorate or direct behavior, is necessary for conditioned approaches to environmental stimuli, mediates augmentation of conditioned reinforcement by psychostimulants and instrumental responding, and the PIT effect. This had led to the assertion that the NAc mediates the motivational influence of Pavlovian CSs, termed incentive salience (Berridge and Robinson, 1998) or incentive value (Dickinson et al., 2000), which greatly influences the ongoing pursuit of rewards.

**The basolateral amygdala**

While the amygdala has classically been discussed in the regulation of negative affect and emotional processing, it has become increasingly evident that this structure is critically involved in the neural processing of positive affect and reward (Murray, 2007). Like the NAc, the amygdala is a heterogenous structure comprised of subregions that exhibit robust differences in anatomy and function (Sah et al., 2003). Two of these structures, the central nucleus of the amygdala (CeN) and the basolateral amygdala (BLA) have been implicated in
reward processing. This dissertation is primarily interested in the contributions of the BLA, which includes the lateral, basal and accessory basal nuclei, to reward processing.

**BLA cellular and chemical composition**

Neuronal cell types within the BLA have been well-characterized. The predominant cell population can be described as the pyramidal-like projection neurons, which comprise roughly 70% of the population (McDonald, 1982, 1984). These cells have pyramidal soma, in the range of 15-20 microns, with several dendrites emanating from the soma (McDonald, 1982). The axons of these cells originate from the soma or the initial portion of the primary dendrite and extend several collaterals within the local region, before projecting to the efferent bundles of the amygdala. These pyramidal-like neurons are largely glutamatergic neurons, and form the primary outputs of the BLA. Electrophysiological studies have demonstrated that these projection neurons exhibit low levels of spontaneous activity, and can be characterized by broad action potentials (Pare et al., 1995; Pare and Gaudreau, 1996).

The second cell population within the BLA is an interneuron, characterized by smaller soma than the projection neurons, while also displaying fewer dendrites with a spherical dendritic field (McDonald, 1982). Furthermore, they produce dramatic axonal collaterals branching several times. These interneurons have been demonstrated to be GABAergic, and express either parvalbumin or calbindin/calretinin (McDonald, 1994, 1997; Kemppainen and Pitkanen, 2000), suggesting potential subclassification within this group. The functional contributions of these interneurons remains a question within the literature.

**Afferent and efferent projections**

The rodent BLA receives numerous afferent projections from a diverse set of brain regions, including cortical and subcortical regions (Pitkanen et al., 2000; Sah et al., 2003).
Briefly, cortical inputs to the BLA arise primarily from layer V pyramidal neurons, and are glutamatergic in nature (McDonald, 1998). The BLA receives inputs across both sensory-specific and polymodal cortical projections (McDonald, 1998; Sah et al., 2003). These include, respectively, information from the gustatory, visceral, somatosensory, auditory, and visual cortices in addition to perirhinal, frontal and hippocampal inputs (Pitkanen, 2000). There are also sparser afferent projections from thalamic nuclei, the hypothalamus, and regions of the midbrain, including a dopaminergic input (Pitkanen, 2000; Pitkanen et al., 2000).

The BLA has substantial efferent projections to corticolimbic circuitry, as well as to other regions of the amygdala. Briefly, the BLA provides glutamatergic projections to regions of the hippocampus and perirhinal cortex (Pitkanen, 2000). The BLA also has strong reciprocal connections with regions of the PFC including the medial and orbital subregions (Pitkanen, 2000). Important for our discussion is the strong striatal projection of the BLA to the NAc (Kelley et al., 1982; McDonald, 1991). This glutamatergic projection has been demonstrated to be topographically organized (McDonald, 1991). In addition to these direct projections to the NAc, numerous studies have demonstrated an indirect pathway to the NAc via PFC neural activity (McGinty and Grace, 2008, 2009b, a).

**The BLA and reward**

As noted above, the BLA has been implicated for a long time in aversive and emotional learning. Lesions of the BLA will severely disrupt the ability of rats to develop conditioned aversions, measured by freezing or fear-potentiated startle (Ledoux, 2000; Pare et al., 2004). However, increasing evidence suggests that the BLA is more than simply a “fear” structure, but rather a critical substrate that mediates associative processes across both
appetitive and aversive conditions (Murray, 2007). This being said, not all associative processes are dependent on an intact BLA. This section will review the current conceptions of the contribution of the BLA to reward processes.

Early studies on the role of the BLA in appetitive conditioning revealed that rats with BLA lesions were able to acquire normal Pavlovian conditioned responses to a CS that was paired with food (Hatfield et al., 1996). Likewise, a simple conditioned taste aversion is readily learned despite a lesioned BLA (Dunn and Everitt, 1988; Hatfield et al., 1996). Together, these demonstrate that an intact BLA is not necessary for the direct associations between a CS and the response. However, in these same paradigms, BLA lesioned rats displayed an inability to adjust their conditioned responding appropriately when the outcome was devalued (Hatfield et al., 1996), suggesting that the BLA was necessary for the ability to use the CS to access the value of the US, and subsequently adjust responding accordingly (Everitt et al., 2000; Cardinal et al., 2002).

Examination of other associative deficits as a result of BLA manipulation have supported this hypothesis. For example, BLA lesioned rats are unable to acquire second-order conditioning, are unable to acquire responding under second-order instrumental tasks (Everitt et al., 1989; Whitelaw et al., 1996), and cannot use a CS as a conditioned reinforcer (Cador et al., 1989; Burns et al., 1993). As noted above, BLA lesioned rats have impairments in the expression of outcome-specific reward devaluation (Johnson et al., 2009), which also requires the ability to assess the affective value of the US. BLA lesioned animals also exhibit robust deficits on conditioned place preference tasks (Everitt et al., 1991). BLA activity is also required for conditioned cues to evoke responding following extinction (Fuchs and See, 2002; See, 2002; Yun and Fields, 2003; Fuchs et al., 2006). Recent evidence has
demonstrated that the BLA representation of the US is not simply reflective of sensory representations of the US, but more specifically reflect the sensory representation of cues that evoke motivational or affective significance at the time of conditioning (Dwyer and Killcross, 2006). Importantly, BLA lesioned rats are not completely insensitive to changes in outcome value, as preferences between foods is maintained (Rolls and Rolls, 1973; Murray et al., 1996).

Given that instrumental responding is not readily disrupted by manipulations of the BLA (Burns et al., 1999; Parkinson et al., 2000; Balleine et al., 2003), it is likely that BLA regulation of instrumental actions are mediated through cue-evoked representations of the outcomes. This is supported by evidence that BLA lesioned rats are impaired at conditioned reinforcement (Cador et al., 1989; Burns et al., 1993; Burns et al., 1999) and outcome-specific forms of PIT (Blundell et al., 2001). It is becoming clearer then, that the BLA is critical for maintaining the associations between environmental stimuli and the affective representations that these stimuli assign to the US (Everitt et al., 2000; Cardinal et al., 2002; Everitt et al., 2003). As such, signaling within the BLA is ideally situated to facilitate motivational responses to environmental stimuli, and mediate adaptive responding through affective representations of outcomes.

**Neurochemical and neurophysiological investigations of NAc signaling**

Although pharmacological and lesion studies have provided numerous insights concerning the role of NAc signaling in reward processes, these methodological approaches do not always provide a clear image of the ongoing signaling that occurs while animals are engaged in behavior. More recently, both *in vivo* electrophysiological and electrochemical methods have been applied to investigate the role of the NAc in food and drug seeking
behaviors. These approaches provide a unique perspective of NAc function because they examine the precise correlation between neural or neurochemical activity and behavioral events, with a temporal resolution that is lacking with other techniques.

For example, while microdialysis investigations have long reported increases in NAc dopamine levels during reward-seeking behaviors and the delivery of rewards (Di Chiara, 2002), these measurements are often made over minutes, and lack the temporal resolution necessary to associate dopamine with precise (real-time) behavioral observations. Recently, the ability to measure dopamine release on a physiologically and behaviorally relevant timescale has led to a focus on rapid NAc dopamine release events (Garris et al., 1999; Phillips et al., 2003b; Robinson et al., 2003). Using fast-scan cyclic voltammetry (FSCV) which allows for subsecond detection of dopamine concentration, research from our laboratory has demonstrated that operant responses for a sucrose reward were associated with robust increases in NAc dopamine concentration (Roitman et al., 2004; Stuber et al., 2004; Stuber et al., 2005a). Similar dopamine signals have also been observed in male rats during exposure to and approach towards receptive females (Robinson et al., 2001). Furthermore, recent results indicate that subsecond increases in NAc dopamine concentration are seen in response to an appetitive sucrose solution, but aversive quinine solutions evoke a pause in dopamine release (Roitman et al., 2008).

In vivo electrophysiological methods have also been applied to investigate the role of the NAc in food and drug seeking behaviors. These approaches provide a unique perspective of NAc function because they elucidate the precise correlation between neural activity and behavioral events. Using these techniques, researchers have demonstrated that NAc neurons exhibit patterned changes in activity (increases and decreases in firing rate) before, during,
and after the completion of instrumental responses for food and drug rewards as well as during the presentation of cues that signal the availability of rewards (Carelli and Deadwyler, 1994; Peoples et al., 1997; Carelli et al., 2000; Nicola and Deadwyler, 2000; Carelli, 2002, 2004; Nicola et al., 2004b; Peoples et al., 2004; Day et al., 2006). However, these patterns of cellular activity are not homogenous. In fact, some NAc cells display enhanced activation before a lever press, while the activity of other neurons may increase or decrease immediately after the lever press (Carelli and Deadwyler, 1994, 1997; Carelli, 2002).

Recent studies have attempted to isolate reward-specific NAc activity through dissociations in the experimental design of reward tasks. For example, in one study NAc cellular activity was monitored while naive rats received experimenter-controlled intra-oral infusions of rewarding sucrose (Roitman et al., 2005). Consistent with other reports (Nicola et al., 2004a; Taha and Fields, 2006), the predominant response of NAc neurons to sucrose infusions was a decrease in activity. However, the same neurons exhibited opposite responses when an aversive quinine solution was delivered intra-orally. One hypothesis suggests that inhibitions observed during reward delivery occur among GABA-containing NAc neurons that project to important motor areas in the VP. Through the disinhibition of target neurons, such a change in activity could provide a gating signal for consummatory behaviors (Nicola et al., 2004a; Roitman et al., 2005; Taha and Fields, 2006). Two recent pieces of evidence support this hypothesis. One recent study found that individual VP neurons exhibit increases in firing rate during consumption of a rewarding sucrose solution, the precise prediction of the gating hypothesis (Tindell et al., 2006). The second study demonstrated that within the NAc, the neural activity is not static, but can be conditioned. Specifically, a previously appetitive stimulus (saccharin), that evoked consummatory orofacial movements, was paired
with cocaine, and became aversive, both in the behavioral rejection, and the NAc neural correlate response (Wheeler et al., 2008). Importantly, there is a relatively small subset of NAc neurons exhibit increases in activity when sucrose rewards are delivered (Taha and Fields, 2005). However, the magnitude of activation varies based on the concentration of sucrose, indicating that these neurons encode the palatability of a food reward instead of reward delivery or consumption. Thus, NAc neurons seemingly process remarkably different types of reward-related information, which could reflect the dual role of the NAc in both the appetitive and consummatory phases of reward behaviors (Nicola et al., 2004a).

Additional experimental designs have allowed for precise characterization of NAc cellular activity during conditioned reward tasks. In one experiment, rats were repeatedly exposed to a CS that was always followed by a sucrose reward as well as a control stimulus that was not paired with a reward (Day et al., 2006). Across several conditioning sessions, rats gradually developed selective conditioned approach responses towards the reward predictive cue, but not towards the unpaired cue. Consistent with another recent study that employed a similar paradigm (Wan and Peoples, 2006), a majority of NAc neurons exhibited marked changes (increases and decreases) in firing rate during presentation of the reward-paired CS in well-conditioned rats. Of these cells, roughly half responded with a prolonged inhibition, while the other half were activated by the presence of the cue, again suggesting that individual neurons within the NAc may operate as a part of microcircuits with distinct functional responsibilities (Carelli and Wightman, 2004). Another study examined NAc cellular activity while rats were engaged in a discriminative stimulus task (Nicola et al., 2004a; Yun et al., 2004b). This study demonstrated that subsets of NAc neurons exhibit robust increases or decreases in activity in response to DS presentation, and that these signals
carry motivational significance (Nicola et al., 2004a). Importantly, recent evidence indicates that these cue-evoked responses among NAc neurons are dependent upon the activity of dopamine neurons (Yun et al., 2004b).

These studies described above are meant as a brief introduction to some of these neurochemical and neurophysiological investigations. For more in-depth discussion please refer to each individual chapter.

**BLA-NAc circuit and reward**

As reviewed above, both the NAc and the BLA contribute to reward processing and adaptive behaviors guided towards them. However, the functional link between these two structures has only recently been examined. Recent evidence has demonstrated a unique contribution of the BLA-NAc circuit in regulating conditioned cue-evoked responding for both food (Setlow et al., 2002b) and cocaine (Di Ciano and Everitt, 2004). These studies utilized disconnection techniques, by inactivating contralateral NAc and BLA within the same animal to demonstrate the necessity of this path to the behavior. Specifically, these studies demonstrated that without the BLA-NAc pathway, animals were unable to respond on a second-order conditioning task for either natural (Setlow et al., 2002b) or cocaine reinforcer (Di Ciano and Everitt, 2004). Additionally, recent evidence has shown that DS-maintained instrumental responding is also attenuated as a result of this BLA-NAc disconnection (Ambroggi et al., 2008).

Neurophysiological evidence has long demonstrated the nature of the connection between the BLA and NAc demonstrating that stimulation of BLA efferents evoke excitatory responses in NAc neurons (O'Donnell and Grace, 1995; Floresco et al., 2001c; Charara and Grace, 2003; Ambroggi et al., 2008; McGinty and Grace, 2008). Extracellular recordings
have also shown that neurons in the BLA exhibit robust phasic response profiles to reward associated cues (Carelli et al., 2003; Sugase-Miyamoto and Richmond, 2005; Paton et al., 2006; Tye and Janak, 2007; Tye et al., 2008). Furthermore, a BLA-dependent facilitation of dopamine within the NAc has been shown, however only in anesthetized animals (Floresco et al., 1998), or using longer temporal measurements (Howland et al., 2002) which may not reflect the rapid time-scale of behaviorally significant events. Additionally, studies in behaving animals have yielded inconclusive evidence that this modulation occurs in a behaviorally relevant manner (Louilot and Besson, 2000; Ahn and Phillips, 2003)

**Goals of this dissertation**

As reviewed here, the NAc and its afferent projections have been implicated in aspects of adaptive responding for rewards. Furthermore, with advances in neurochemical and neurophysiological techniques, real-time characterization of NAc signaling during sophisticated behavioral tasks have allowed for novel insights regarding the role of the NAc in these reward processes. However, the functional role that afferent projections play in mediating NAc signaling remains largely unknown. Previous investigations from our laboratory and others have found that NAc neurons exhibit time-locked phasic changes in activity during the presentation of reward-paired cues (Nicola et al., 2004b; Roitman et al., 2005; Day et al., 2006) and operant responses to obtain rewards (Carelli et al., 2000; Carelli and Wightman, 2004). Likewise, dopamine release within the NAc is increased following presentation of both conditioned (Phillips et al., 2003b) and discriminative stimuli (Roitman et al., 2004), as well as during uncued operant responses (Phillips et al., 2003b; Roitman et al., 2004; Stuber et al., 2004; Stuber et al., 2005a). Given that NAc activity is a result of integrated inputs from limbic structures such as the BLA, and these inputs are apposed to
dopaminergic terminals, it is of great import to determine the specific contribution that these neural substrates have in mediating NAc signaling during reward seeking behavior. Thus, the following studies seek to elucidate the behavioral role of neurochemical and neurophysiological signals in the NAc by assessing subsecond NAc dopamine release and NAc cellular activity during a variety of behavioral tasks, coupled to circuit-level pharmacological manipulation of neural activity within the BLA. Together, these studies provide a significant advance in our understanding of the functional regulation, and behavioral relevance of BLA-NAc signaling.

Specific Aims:

1. Determine the contribution of BLA activity to phasic dopamine signaling within the NAc core during cued-instrumental responding for sucrose. Previous work from our laboratory has demonstrated robust phasic dopamine release in the NAc during the presentation of reward-predictive stimuli (Roitman et al., 2004; Day et al., 2007). Although, phasic dopamine transients within the NAc arise primarily from activity of dopamine neurons located in the ventral tegmental area (VTA) (Sombers et al., 2009), stimulation of the BLA can elicit dopamine release within the NAc independent of VTA activation (Floresco et al., 1998). Given the dramatic role of D1 receptor activation in synaptic plasticity and learning mechanisms (Nicola et al., 2000; Pawlak and Kerr, 2008), subtle changes in dopamine release could dramatically alter learned associations at glutamatergic synapses within the NAc. In these experiments, we will use fast-scan cyclic voltammetry (FSCV) to measure rapid changes in NAc dopamine concentration while rats engage in a discriminative stimulus task for sucrose reward. Additionally, to assess how BLA activity modulates phasic
dopamine signals we will pharmacologically inactivate the BLA. As cues will be presented well before the opportunity to respond, this design will enable us to dissect whether BLA activity regulates NAc dopamine signaling associated with cue-onset, the instrumental response, or reward delivery. Results from these experiments have been published (see; Jones, J.L., Day, J.J., Aragona, B.J., Wheeler, R.A., Wightman, R.M., Carelli, R.M. (2009) Basolateral amygdala modulates terminal dopamine release in the nucleus accumbens and conditioned responding. *Biological Psychiatry* (ePub ahead of print)).

2. **Determine the contribution of BLA activity to neurophysiological signals within the NAc during cued-instrumental responding for sucrose.** Environmental stimuli that consistently predict rewards can develop biological salience and promote reward-seeking behavior in a manner that is both NAc and dopamine dependent (Dickinson et al., 2000; Everitt et al., 2001; Robbins and Everitt, 2002; See, 2002; Kalivas and McFarland, 2003; Yun et al., 2004a; Yun et al., 2004b). This aim will employ multi-unit extracellular electrophysiology to examine the neurophysiological responses of NAc neurons during a discriminative stimulus task for a natural reward, sucrose. Additionally, we will examine the specific contribution of the BLA to these NAc cellular responses by recording NAc cell firing before and during pharmacological inactivation of the BLA.

3. **Determine the contribution of BLA activity to neurophysiological signals in the NAc during cocaine self-administration.** Distinct subsets of NAc neurons exhibit patterned cell firing during cocaine self-administration (Carelli and Deadwyler, 1994, 1997), and are also responsive to cocaine associated conditioned stimuli (Carelli, 2000; Carelli and Ijames,
2001). Additionally, we have shown that neurons within the BLA exhibit phasic response profiles during cocaine self-administration and are also activated by cues previously associated with cocaine delivery (Carelli et al., 2003). Here, we recorded NAc cell firing during cocaine self-administration, and relative to cocaine-associated cue presentation, before and during pharmacological inactivation of the BLA. Our findings provide insight into how BLA activity functionally modulates neurophysiological responses in the NAc during cocaine self-administration in a manner similar to sucrose reinforcement (Aim 2). Furthermore, we will examine if BLA input drives NAc cell firing to cocaine-associated cues.
CHAPTER 2

BASOLATERAL AMYGDALA MODULATES TERMINAL DOPAMINE RELEASE IN THE NUCLEUS ACCUMBENS AND CONDITIONED RESPONDING

Data within this chapter have been published in Biological Psychiatry, x(x) xxx-xxx (2009).

ABSTRACT

It has been shown that phasic (subsecond) dopamine signaling within the nucleus accumbens (NAc) is time-locked to salient environmental cues. However, the role of associative neural substrates such as the basolateral amygdala (BLA) in regulating phasic dopamine release in the NAc, particularly during conditioned reward tasks, remains unknown. Here, we used pharmacological inactivation (GABA agonists baclofen/muscimol; BM) of the BLA in conjunction with in vivo voltammetric recordings to examine the contribution of BLA activity to rapid dopamine signaling in the NAc core during a cue-instrumental task for sucrose. Rats learned to discriminate two cues, a discriminative stimulus (DS) that signaled sucrose reward contingent upon a lever press and a non-associated cue (NS) that signaled another spatially distinct lever that was never rewarded. DS onset evoked a significant increase in dopamine release within the NAc core. DS evoked dopamine release was significantly greater than the NS that persisted throughout the instrumental response. Importantly, GABA agonist microinfusion into the ipsilateral BLA decreased DS-evoked approach responses and selectively attenuated phasic dopamine release elicited by the DS. Dopamine release related to the instrumental response or evoked by electrical stimulation of the ventral tegmental area (VTA) was not altered by BLA
inactivation. Thus, the BLA contributes to phasic DA signaling within the NAc related to reward-associated stimuli, and may facilitate the attribution of incentive value to environmental cues.
INTRODUCTION

The ability of an organism to successfully pursue, procure and consume rewards is a critical determinant of survival. Organisms learn to assign value to relevant environmental stimuli that are associated with successful goal-directed action. These associations can produce marked changes in an organism’s ability to direct and guide future decisions. Numerous lines of research have demonstrated that these associative processes are mediated by a distributed network of brain nuclei including the nucleus accumbens (NAc) and its midbrain dopaminergic input from the ventral tegmental area (VTA). Furthermore, dysfunction of these associative processes within this network appears to underlie aspects of compulsive drug-seeking and addiction (Berke and Hyman, 2000; Kelley, 2004a; Hyman et al., 2006).

The importance of NAc dopamine to reward is clear (Wise, 2004), particularly in the acquisition and expression of certain learned associations (Smith-Roe and Kelley, 2000; Di Ciano et al., 2001), responding to reward-paired cues (Nicola et al., 2005), and reward-related decision making (Phillips et al., 2007). VTA dopamine neurons that project to the NAc signal critical determinants of reward value (Schultz, 2007), and we have demonstrated terminal dopamine release within the NAc during the presentation of rewards and reward-predictive stimuli (Roitman et al., 2004; Day et al., 2007). Nevertheless, very little is known regarding neural substrates that regulate NAc phasic dopamine release during behavior.

Phasic dopamine release arises primarily from burst-firing of VTA dopamine neurons (Sombers et al., 2009), and both its development (Owesson-White et al., 2008; Stuber et al., 2008) and disruption (Zweifel et al., 2009) correlate with changes in learned reward-related behaviors. Phasic dopamine signals are critical for motivated behaviors as VTA-mediated
dopamine transmission within the NAc is both necessary for behavioral responses to reward-predictive cues (Nicola et al., 2005) and sufficient for reward-related conditioning (Tsai et al., 2009). However, post-synaptic signals in NAc neurons are not driven by dopamine alone (Nicola et al., 2000), but through a complex integration of glutamatergic afferent input with concurrent dopaminergic signals (Nicola et al., 2000; Reynolds et al., 2001). As such, a critical question is whether glutamatergic afferents influence NAc dopamine release at the terminal level to functionally alter responding to reward-predictive stimuli.

The basolateral amygdala (BLA), a structure linked with associative learning (Everitt et al., 2003; Pare et al., 2004), is anatomically positioned to modulate the terminal release of dopamine (Kelley et al., 1982). Furthermore, BLA-NAc interactions are critical for reward-seeking (Setlow et al., 2002b; Di Ciano and Everitt, 2004), and BLA activity can significantly alter NAc cellular responding (Floresco et al., 2001c; Ambroggi et al., 2008). As such, it has been proposed that BLA activity contributes to phasic dopamine release within the NAc (Phillips et al., 2003a). However, BLA-dependent facilitation of dopamine within the NAc has been shown in anesthetized animals (Floresco et al., 1998), or during longer temporal measurements (Howland et al., 2002) which may not reflect the rapid time-scale of behaviorally significant events. Further, studies in behaving animals have been unable to confirm that this modulation occurs in a behaviorally relevant manner (Louilot and Besson, 2000; Ahn and Phillips, 2003).

Here, we used fast-scan cyclic voltammetry within the NAc coupled to microinfusion into the BLA of GABA_A and GABA_B agonists (muscimol 0.03 nmol and baclofen 0.3 nmol in 0.3µL) (McFarland and Kalivas, 2001) to determine the functional contribution of the BLA to phasic dopamine release in the NAc during a discriminative-stimulus instrumental
task. We hypothesized that disruption of glutamatergic BLA inputs to the NAc core would decrease cue-evoked dopamine terminal release and motivated responding. Our results provide a critical characterization of afferent modulation of phasic dopamine signaling at the terminal level in the NAc, and demonstrate a functionally relevant mechanism by which the BLA can selectively facilitate responding to motivationally salient events.
METHODS

Subjects

Male Sprague Dawley rats (Harlan Sprague Dawley, Indianapolis, IN) aged 90–120 d and weighing 260–350 gm were used as subjects and individually housed with a 12:12 light:dark cycle. All experiments were conducted between 8:00 am and 6:00 pm. Bodyweights were maintained at no less than 85% of pre-experimental levels by food restriction (10–15 gm of Purina laboratory chow each day, in addition to approximately 1 gm of sucrose consumed during daily sessions). This regimen was in place for the duration of behavioral testing, except during the post-operative recovery period, when food was given ad libitum. All procedures were approved by the University of North Carolina at Chapel Hill Institutional Animal Care and Use Committee.

Surgery

Rats were surgically prepared for voltammetric recordings using established procedures as described previously (Day et al., 2007). After establishing an anesthetic plane with ketamine hydrochloride (100mg/kg, intramuscular) and xylazine hydrochloride (20 mg/kg, intramuscular), rats were placed in a stereotaxic frame. A guide cannula was stereotaxically positioned in the NAc core (1.3-1.5 mm anterior, 1.3 mm lateral from bregma) and a bipolar stimulating electrode in the VTA (5.2 mm posterior, 1.0 mm lateral from bregma, 7 mm ventral from brain). A Ag/AgCl reference electrode was placed contralateral to the stimulating electrode in the left forebrain. A detachable micromanipulator containing a glass-sealed carbon-fiber electrode (75–100 µm exposed tip length, 7 µm diameter, T-650; Amoco, Greenville, SC) was inserted into the guide cannula, and the electrode was lowered into the NAc core. The bipolar stimulating electrode was then lowered
in 0.2 mm increments until electrically evoked dopamine release was detected at the carbon-fiber electrode in response to a stimulation train (60 biphasic pulses, 60 Hz, 120 µA, 2 ms per phase). The stimulating electrode was then fixed with dental cement and the carbon-fiber electrode was removed. An additional guide cannula (Plastics One) was implanted 1 mm above the BLA (2.7-3.1 mm posterior, 4.9-5.0 lateral from bregma; 7.7mm from ventral from skull).

Fast-scan cyclic voltammetry

Following surgery, animals were allowed one week to recover pre-surgery body weight. Food intake was then reduced to ensure motivation during conditioning. To collect electrochemical data on the test day, a new carbon-fiber electrode was placed in the micromanipulator and attached to the guide cannula. The carbon-fiber electrode was then lowered into the NAc core. The carbon-fiber and Ag/AgCl electrodes were connected to a head-mounted voltammetric amplifier attached to a commutator (Crist Instrument Company, Hagerstown, MD) at the top of the experimental chamber. All electrochemical data were digitized and stored using computer software written in LabVIEW (National Instruments, Austin, TX). To minimize current drift, the carbon-fiber electrode was allowed to equilibrate for 30−45 min prior to the start of the experiment.

The potential of the carbon-fiber electrode was held at −0.4 V versus the Ag/AgCl reference electrode. Voltammetric recordings were made every 100 ms by applying a triangular waveform that drove the potential to +1.3 V and back at a rate of 400 V/s. The application of this waveform causes oxidation and reduction of chemical species that are electroactive within this potential range, producing a change in current at the carbon-fiber. Specific analytes (including dopamine) are identified by plotting these changes in current
against the applied potential to produce a cyclic voltammogram (Heien et al., 2004). The stable contribution of current produced by oxidation and reduction of surface molecules on the carbon-fiber was removed by using a differential measurement (i.e., background-subtraction) between a time when such signals were present but dopamine was not. For data collected during the behavioral session, this background period (500 ms) was obtained during the baseline window (5 s prior to cue onset), was obtained at the minima for the current signal during this window. Theoretically, this background subtraction should not remove any dopamine, because the background was explicitly selected for the absence of fast dopamine signals. Following equilibration, dopamine release was electrically evoked by stimulating the VTA (24 biphasic pulses, 60 Hz, 120 µA, 2 ms per phase) to ensure that carbon-fiber electrodes were placed close to release sites. The position of the carbon-fiber was secured at the site of maximal dopamine release. Experiments began when both stimulated dopamine release was detected, and spontaneously occurring transients could be detected at the electrode. During the behavioral sessions, experimental and behavioral data were recorded with a second computer, which translated event markers to be time-stamped with electrochemical data. VTA stimulation was repeated following the experiment to verify electrode stability and ensure that the location of the electrode could still support dopamine release.

FSCV is an electrochemical measurement technique that takes advantage of the electroactive nature of specific analytes such as dopamine, which undergo an oxidation and reduction reaction in response to changes in voltage. Here, a carbon fiber electrode is encased in a glass pipette and pulled to a sharp tip, such that only 75-100 mm of the carbon fiber is exposed. Measurements are made by ramping the voltage of the electrode to a level
that oxidizes dopamine (to dopamine-ortho quinone) and then back to its original potential, which reduces dopamine-ortho quinone back to dopamine. This change in applied voltage typically takes 10ms, and is repeated every 100ms. The result of each scan is a large faradaic current that results from oxidation and reduction of electroactive chemical species near the electrode as well as changes on the surface of the carbon fiber electrode (Kawagoe et al., 1993). This current can be detected at the exposed carbon fiber and plotted against the applied potential to produce a cyclic voltammogram, which can be subtracted from other cyclic voltammograms to provide information on how the current changed over time. As electroactive species oxidize and reduce at different voltages, background-subtracted cyclic voltammograms also provide information on the specific analyte in question (Heien et al., 2004; Heien et al., 2005), allowing dissociable measurement of ascorbate, serotonin, DOPAC, and pH (Cahill et al., 1996; Bunin and Wightman, 1998; Heien et al., 2004). Thus, FSCV provides subsecond (100ms) temporal resolution in detecting changes in dopamine at terminal regions, and has recently been applied successfully to real-time measurement of dopamine release in behaving animals (Robinson et al., 2002; Phillips et al., 2003c; Phillips et al., 2003b; Roitman et al., 2004).

**Signal identification and separation**

After in vivo recordings, dopamine release evoked by VTA stimulation was used to identify naturally occurring dopamine transients using methods described previously (Heien et al., 2004; Heien et al., 2005). Stimulation of the VTA leads to two well-characterized electrochemical events: an immediate but transient increase in [DA] and a delayed but longer-lasting basic pH shift. To separate these signals, a training set was constructed from representative, background-subtracted cyclic voltammograms for dopamine and pH. This
training set was used to perform principal component regression on data collected during the behavioral session. Principal components were selected such that at least 99.5% of the variance in the training set was accounted for by the model. All data presented here fit the resulting model at the 95% confidence level. After use, carbon-fiber electrodes were calibrated in a solution of known [DA] to convert observed changes in current to differential concentration.

**Behavioral Task**

One week after surgery, animals were trained on a discriminative stimulus task (8-12 days) followed by a single test session (Figure 2.1). Rats discriminated two tones associated with spatially distinct levers. One tone (2750 Hz or 1000 Hz; discriminative stimulus; DS) was presented for 500 ms accompanied by illumination of a cue-light above lever 1. Three seconds after DS onset lever 1 was extended; depression resulted in lever retraction, termination of the cue-light, and delivery of a sucrose pellet (45mg). A second distinctive tone (alternate Hz; non-associated stimulus; NS) was presented for 500 ms with the associated cue-light above lever 2; 3 s later lever 2 was extended. Depression of this lever resulted in lever retraction, cue-light termination but no reward. Subsequent DS or NS trials were random and presented on a variable interval 15s schedule.

Following training, a single test session was completed in 3 phases. In phase 1, 15 sucrose pellets were randomly delivered on a variable interval schedule (mean=30s), to replicate previous findings (Day et al., 2007). Phase 2 consisted of unilateral infusion of either vehicle (VEH; 0.3µL of sterile saline 0.9% NaCl) or baclofen/muscimol (BM; 0.3/.03nmol in 0.3µL VEH; Sigma Aldrich) into the BLA followed by five VTA stimulations and a 60 trial session (30 DS, 30 NS; randomized order). A recovery phase followed. Phase
3 consisted of infusion of the alternate infusate into the BLA, stimulation collection, another 60 trial session and a subsequent recovery phase.

![Figure 2.1. Task and experimental protocol](image)

Animals were semi-randomly presented one of two trial types (DS or NS), each distinguished by a unique auditory tone, and subsequent presentation of a spatially distinct lever (L1 or L2). Each response on the DS lever resulted in sucrose pellet delivery (FR1, schedule of reinforcement). Responses on the NS lever were never rewarded. Triangles denote lever presses. The variable ITI (vITI) averaged 15s. (b) The test session was divided into two phases in which delivery of either VEH or BM were microinfused into the BLA. Following Infusion 1, animals were given 60 trials (30 DS, 30 NS) and dopamine release was measured. Upon completion, a recovery period was initiated followed by Infusion 2, completion of a second test session, and subsequent recovery period.

**Data analysis**

Several behavioral measures were examined: the DS and NS approach response ratios, the DS response latency, and the number of overall responses on the DS- and NS-levers. Approach responses were analyzed through video analyses, wherein an approach was defined as a directed movement or orienting of the animals head into the cue-lever region of the chamber (2in. x 2in. surrounding lever) during the DS or NS presentation, but prior to the lever presentation. The latency to reach the sucrose delivery well, and the sucrose consumption period (defined as time in which the rats head remained over the sucrose delivery well) were also scored via video analysis. Response latencies and lever presses were recorded via computer. A within-subjects repeated-measures ANOVA was used to
compare lever pressing across training sessions. Bonferroni *post hoc* tests compared replicate means across cue condition. Cue-evoked approach percentages were compared using paired t-tests across VEH and BM conditions. Cue-evoked lever responses were compared using paired t-tests.

Principal component regression was used to extract the dopamine component from the voltammetric data (Heien et al., 2004). Training sets constructed from representative, background-subtracted cyclic voltammograms for dopamine and pH allow for principal component regression on data collected during the behavioral session, described previously (Day et al., 2007). Changes in NAc dopamine concentration ([DA]) were evaluated using a one-way repeated measures ANOVA with Dunnett’s *post hoc* test for multiple comparisons of 100-ms time bins (7s post-cue) to a single baseline window (mean [DA] from 5 s pre-cue onset). The average peak change in [DA] was determined for both DS and NS for each animal and statistically compared via Student’s paired t-tests.

The average peak change in [DA] was determined for the DS, NS, or stimulation for each animal across both VEH and BM treatments (i.e. all trials for a given animal were averaged to provide a single [DA] trace), and this average was statistically compared via Student’s paired t-tests. Differences in [DA] relative to lever extension or lever press were similarly examined by determining the peak change in [DA] in the 2s following onset and comparing it to pre-cue baseline using Student’s paired t-tests. For sucrose consumption, [DA] was averaged across the consumption period, then compared between treatments using Student’s paired t-tests.

Dopamine release events occur independently of any overt behavioral stimuli (Robinson et al., 2002; Stuber et al., 2005b). To determine the effect of BLA activity on the
likelihood of high [DA] release events, every 100 ms sample from each trial for each rat was
time-stamped if it contained a concentration increase of 40 nM or higher. This threshold
represents the average value of spontaneous dopamine release events (Stuber et al., 2005b)
that are a result of burst firing of VTA dopamine neurons (Sombers et al., 2009). Furthermore, this [DA] is within the range of affinities for high-affinity D1 receptor
(Richfield et al., 1989). With these data, the probability of [DA] concentrations exceeding 40
nM was calculated (Prob_{40}). A two-way ANOVA was used to identify main effects of epoch
(baseline versus DS) and treatment (VEH versus BM). Bonferroni post hoc tests for multiple
comparisons were used to identify significant differences within epoch and treatment.

Statistical significance was designated at p < 0.05. All statistical analyses were
carried out using Graphpad Prism 4.0 for Windows (Graphpad Software) or SPSS version
17.0 for Windows (SPSS).

Histological verification of electrode placement

Upon completion of each experiment, rats were deeply anesthetized with a
ketamine/xylazine mixture (100 mg/kg and 20 mg/kg, respectively). In order to mark the
placement of electrode tips, a 50–500 µA current was passed through a stainless steel
electrode for 5 seconds. Brains were removed and then placed in 10% formalin, and brains
were removed. After post-fixing and freezing, 50 µm coronal brain sections were mounted on
microscope slides. The specific position of individual electrodes was assessed by visual
examination of successive coronal sections. Placement of an electrode tip within the NAc
was determined by examining the relative position of observable reaction product to visual
landmarks (including the anterior commissure and the lateral ventricles) and anatomical
organization of the NAc represented in a stereotaxic atlas (Paxinos and Watson, 2005).

Figure 2.2 provides a histological representation of electrode and cannula placements.

![Figure 2.2. Histological representation of electrode and cannula placement. Top diagrams show the NAc core placements of the carbon-fiber electrode at recording. Bottom illustrates the electrode tip of the microinjector placed into the BLA cannula. Green circles represent behavioral controls of infusion sites outside the BLA.](image)
RESULTS

Acquisition of stimulus-controlled behavior

Rats learned the discriminative stimulus task over 8-12 sessions. A two-way repeated measures ANOVA of the final six sessions revealed a significant main effect of session \( (F(5,60) = 12.52; \ p<0.0001) \), cue \( (F(1,60) = 42.69; \ p<0.0001) \) and a significant interaction of session x cue \( (F(5,60) = 4.46; \ p=0.002) \) on the percentage of cue-trials with an instrumental response (Fig. 2.3). *Post hoc* comparisons revealed a significant difference between percentage of cue-trials with an instrumental response over the final three days of training \( (p<0.05) \). The final training response ratios were 100 ± 0 for the DS and 27.8 ± 7.6 for the NS trials.

![Figure 2.3. Acquisition of instrumental responding.](image)

After training, the DS evoked dopamine signals in the NAc core under VEH conditions. Figure 2.4 a and b shows [DA] changes during a single DS and NS trial from a representative animal following VEH treatment. Figure 2.4 c shows the average [DA] for both cues across all animals. The DS produced an immediate increase in [DA] \( (F(6,70) = \)
11.92, p<0.0001). Peak DS-evoked [DA] occurred 560 ± 50 ms after DS onset, reaching an average peak of 83 ± 16 nM and remained elevated throughout the operant response (Bonferroni post-hoc p<0.05), although there was no significant further increase at the lever extension (dashed line), lever press (triangle) or during sucrose consumption. NS presentation evoked a lesser, yet significant increase in [DA] (F_{(6,70)} = 22.61; p<0.0001). The peak NS-evoked [DA] of 38 ± 8 nM occurred 470 ± 40 ms after NS onset, and then rapidly returned to baseline levels, with no significant increase following the lever extension. The latency to peak [DA] was not different between cues (t_{(6)} = 1.87, p=0.11). Importantly, the average peak [DA] evoked by the DS was significantly larger than that evoked by the NS (t_{(6)} =3.55, p=0.012; Figure 2.4d).
Figure 2.4. Reward predictive cues evoke phasic dopamine release in the NAc core. (a) Dopamine release during one representative DS trial. (Top) Voltammetric plot (time x voltage x current) for DS trial and (Bottom) corresponding [DA] determined by principal component analysis. Black bar denotes DS period, dashed line denotes the DS-lever insertion, and the triangle represents the lever press. Inset: cyclic voltammogram taken from peak [DA]. (b) Dopamine release during one representative NS trial. (Top) Voltammetric plot (time x voltage x current) for NS trial and (Bottom) corresponding [DA] determined by principal component analysis. Open bar denotes NS period, and dashed line denotes NS-lever insertion. Inset: cyclic voltammogram taken from peak [DA]. (c) Average Δ[DA] (n=7) relative to DS (black line) or NS (grey line) onset (at time 0) under VEH conditions. Lever extension is denoted by dashed line. Triangle represents mean ± range of DS lever presses across all animals. (d) There was a significant difference in the average peak [DA] between the DS and NS. Error bars show mean ± SEM; * denotes p<0.05.

BLA modulation of DS-evoked behavior and NAc dopamine release

To determine the contribution of BLA activity to DS-evoked behavior and NAc dopamine signaling, we pharmacologically inactivated the BLA with BM. Figure 2.5a shows the percentage of DS trials in which an animal made a conditioned approach response to the cue (i.e., approached the DS-associated lever prior to extension), and paired t-tests demonstrate that BM significantly attenuated DS-evoked approaches (t(8) = 2.456; p=0.04; VEH 95.7 ± 1.75; BM 67.9 ± 11.4). Despite the reduction in cue-evoked approach, BM did not alter the ability to perform the instrumental response once initiated, as the percentage of trials with a DS lever response was unaltered (t(8) = 1.43; p=0.19; VEH 100.0 ± 0.0; BM 95.2 ± 4.8; Figure 2.5b). The latency to respond was not significantly altered following BLA inactivation, although there was an increase (t(8) = 1.95; p=0.09; VEH 489 ms ± 57; BM 832 ms ± 148; data not shown). Furthermore, BLA inactivation did not alter the ability of the rats to consume the sucrose, nor the sucrose consumption duration (t(6) = 1.68; p=0.14; VEH 2.23 s ± 0.23 ; BM 1.88 s ± 0.12).

Concomitant with decreased DS-evoked approaches, we found a significant attenuation of DS-evoked dopamine. Figure 2.5c shows the average [DA] traces aligned to
DS-onset following VEH and BM treatment (left panel) and also aligned to the DS lever press (right panel) across all animals. Paired t-tests showed that BLA inactivation significantly decreased the peak magnitude of DS-evoked [DA] ($t_{(6)} = 2.587; p=0.04$; VEH 83 ± 16 nM versus BM 61 ± 14 nM; Figure 2.5d). However, [DA] following lever insertion (indicated by dashed line; left panel Figure 2.5c) was unaltered by BLA inactivation ($t_{(6)} = 1.07; p=0.32$). Furthermore, Figure 2.5c (right panel) shows the average [DA] traces aligned to the DS-evoked lever press (denoted by triangle) following VEH and BM treatment across all animals. BLA inactivation had no significant effect on peak [DA] following the DS-related lever press ($t_{(6)} = 0.214; p=0.83$; VEH 42 ± 8 nM versus BM 43 ± 10 nM; Figure 2.5e) nor did it alter [DA] during the sucrose consumption period (data not shown; $t_{(6)} = 0.79; p=0.45$).
Figure 2.5. Effects of BLA inactivation on DS-evoked behavioral responding and dopamine release. (a) Percentage of DS trials with a DS-evoked conditioned approach response was significantly attenuated by BM compared to VEH (n=9). (b) Percentage of DS trials with an instrumental response was not altered by BM treatment (n=9). (c) Average Δ[DA] across NAc core recordings (n=7) on DS trials under VEH (black line) and BM (grey line) conditions. The left panel is aligned to DS-onset (at time 0); lever extension denoted by dashed line. The right panel is aligned to the DS lever-press response (triangle). (d) Significant reduction in peak [DA] to the DS following BM treatment compared to VEH (n=7). (e) No significant difference in peak [DA] after the lever press between BLA treatments (n=7). ns denotes p>0.05; * denotes p<0.05.

BLA modulation of NS-evoked behavior and NAc dopamine release

BLA inactivation had no effect on behavioral responses related to the NS. That is, neither the percentage of trials with an NS-evoked conditioned approach response (t_{8} = 1.13;
p=0.29; Figure 2.6a), nor the percentage of NS trials with a lever press were altered following BLA inactivation (t(8) = 0.99; p=0.35; Figure 2.6b). Figure 2.6c shows the average [DA] traces aligned to NS-onset (time 0) following VEH and BM treatment across all animals. No significant difference was observed in the peak [DA] evoked by the NS between VEH or BM treatments (t(6) = 1.73; p=0.13; VEH 38 ± 8 nM BM 27 ± 4 nM; Figure 2.6d).

**Figure 2.6.** Effects of BLA inactivation on behavior and NS-evoked dopamine (a) No significant difference in the percentage of NS trials with a cue-evoked behavioral approach toward the NS lever (n=9). (b) No significant difference in the percentage of NS trials with an associated lever press response (n=9). (c) Average Δ[DA] across NAc core recordings (n=7) on NS trials under VEH (black line) and BM (grey line) conditions. White bar denotes NS period; lever extension denoted by dashed line. (d) No significant difference in peak [DA] to the NS across BLA treatment (n=7). ns denotes p>0.05.

**BLA modulation of VTA-mediated NAc dopamine release**

Next, we examined the mechanism of BLA modulation of dopamine release in the NAc. One possibility is that BLA modulation of NAc dopamine is mediated indirectly through actions on dopamine neurons in the VTA. If so, BLA inactivation should affect VTA
electrically-stimulated dopamine release in the NAc (Sombers et al., 2009). Figure 2.7a shows the average [DA] following electrical stimulation of the VTA (24 biphasic pulses, 60 Hz, 120 µA, 2 ms per phase), during both VEH and BM treatments. The peak VTA-evoked stimulated [DA] was not significantly different between VEH and BM treatments ($t_{(5)} = 0.479, p>0.05$; Figure 2.7b).

Figure 2.7. BLA inactivation does not alter VTA-evoked stimulated dopamine release in the NAc. (a) Average $\Delta$[DA] from NAc recordings (n=6) following VTA electrical stimulation (solid vertical line, time 0) during VEH (black line) or BM (grey line) treatments (b) Average peak [DA] following VTA electrical stimulation is not significantly different across BLA treatments (n=6). Error bars show mean ± SEM; ns denotes $p>0.05$.

Likewise, if BLA modulation of NAc dopamine is mediated via the VTA, a concurrent reduction in the probability of large concentration (>40nM; Prob$_{40}$) dopamine release events in response to the DS should be observed, since dopamine release of this magnitude reflect synchronous burst firing of VTA neurons (Roitman et al., 2008; Sombers et al., 2009). Thus, we assessed the effect of BLA inactivation on the probability of high [DA] release events during two event-related time epochs (baseline: 5s period prior to DS onset; DS: 3s period following DS onset; Figure 2.8a) across both VEH and BM treatments. An example of a representative trial is illustrated in Figure 2.8a. Note the occurrence of one
naturally occurring large dopamine release event during the baseline period as well as a large DS-evoked dopamine release event. Across all animals, two-way repeated measures ANOVA (epoch x treatment) revealed a significant main effect of epoch ($F_{(1,12)} = 62.24; p<0.0001$), but no main effect of treatment ($F_{(1,12)} = 1.16; p=0.30$) on $\text{Prob}_{40}$ (Figure 2.8b) Further, *post hoc* analyses revealed that BLA inactivation did not alter the $\text{Prob}_{40}$ during either the baseline epochs ($p>0.05$; VEH $0.33 \pm 0.05$, BM $0.38 \pm 0.02$) or DS epochs ($p>0.05$; VEH $0.078 \pm 0.06$, BM $0.81 \pm 0.05$). These data indicate that BLA modulation of DS-related NAc dopamine release events are not the result of alterations in burst firing of VTA dopamine neurons.

Figure 2.8. BLA inactivation does not alter high [DA] release probability in the NAc. (a) All trials for one animal aligned to the DS-onset; pink marks denote the lever press. [DA] is represented in the color dimension. (b) A single trial trace of $\Delta$[DA]. Event-related epochs are denoted on the x-axis, divided by the solid vertical line at DS onset (black bar denotes DS period). Dashed horizontal line represents the 40nM threshold. (c) $\text{Prob}_{40}$ significantly differs as a function of event epoch, but neither the baseline nor DS time epochs differ across VEH or BM treatment (n=7). Error bars show mean ± SEM; ns denotes $p>0.05$; * denotes $p<0.05$.

*Experimental controls*

To demonstrate that the results presented are not the result of diffusion of BM from the BLA to neighboring regions, we examined infusion sites located outside the BLA. These sites were located within regions of the piriform cortex, ventral to the target. Figure 2.9
illustrates the effects of BM treatment on the behavioral responding following BLA infusions (n=2). Statistical analyses were not run on these data, but examination of the descriptive statistics suggest there is no effect of piriform treatment on either the percentage of DS trials with an instrumental response (Figure 2.9a; VEH 88.35 ± 8.35, BM 100 ± 0.00), or the DS response latency (Figure 2.9b; VEH 0.64 ± 0.27, BM 0.54 ± 0.05).

Figure 2.9. Effects of inactivation of piriform locations (n=2). (a) The average percentage of DS trials with a lever response. (b) The average DS response latency across both VEH and BM treatments.
DISCUSSION

We examined the contribution of BLA activity to NAc core dopamine during a cued sucrose reinforcement task. The DS, which predicted access to the reinforced lever, evoked significantly higher phasic dopamine than the NS, which predicted access to a non-reinforced lever. Pharmacological inactivation of the BLA selectively attenuated DS-evoked dopamine, concurrent with an attenuation of DS-evoked conditioned approaches. However, dopamine measured following NS-onset, lever extensions, lever presses or during sucrose consumption was unaltered following BLA inactivation. Likewise, VTA electrically-stimulated dopamine release was unchanged following BLA inactivation. These findings demonstrate that BLA activity functionally modulates phasic dopamine release within the NAc, through terminally mediated mechanisms, to facilitate reward-seeking evoked by motivationally salient environmental stimuli.

The importance of NAc dopamine to reward-seeking behavior has been extensively described (Berridge and Robinson, 1998; Everitt et al., 1999). The dopaminergic projection from the VTA to the NAc contributes significantly to cue-evoked behavioral responding (Yun et al., 2004b), and phasic activation of dopaminergic neurons is sufficient to establish a conditioned place preference (Tsai et al., 2009). Burst firing of dopamine neurons drives phasic dopamine release within the NAc (Sombers et al., 2009) and these phasic dopamine signals are time-locked to reward predictive cues within the NAc core (Day et al., 2007). Our data are consistent with previous findings, as we show DS-evoked dopamine release that was significantly higher than NS-related release (Figure 2.4). While our measurements were made following learning, the differential behavioral responding to the DS and NS is consistent with prior studies showing that the development of cue-evoked phasic dopamine
release correlates with successful learning of an appetitive task (Stuber et al., 2008) and disruption of phasic dopamine release can selectively attenuate the acquisition of tasks guided by cues (Zweifel et al., 2009).

However, the primary goal of this study was to determine the functional contribution of BLA activity to ongoing phasic dopamine release within the NAc core. We demonstrate that transient unilateral reduction of BLA activity causes a selective attenuation in the magnitude of DS-evoked dopamine (Figure 2.5). Previous findings have demonstrated that pre-synaptic glutamate receptors (Gracy and Pickel, 1996; Tarazi and Baldessarini, 1999) can mediate dopamine release in the striatum in slices (Krebs et al., 1991b; Krebs et al., 1991a). Further, in anesthetized preparations (Floresco et al., 1998) and awake microdialysis tests (Howland et al., 2002), stimulation of the BLA produces terminally mediated, glutamate-dependent increases in NAc dopamine. BLA neurons are activated by emotionally salient stimuli (Schoenbaum et al., 1999; Carelli et al., 2003; Paton et al., 2006; Tye and Janak, 2007) which in turn drives post-synaptic excitatory responses in NAc neurons (O'Donnell and Grace, 1995; Floresco et al., 2001c; Ambroggi et al., 2008). Our findings indicate that BLA activation to cues, in addition to driving post-synaptic firing, augments concurrent VTA-mediated phasic dopamine release within the NAc.

Furthermore, our findings support the hypothesis that BLA modulation of dopamine signaling occurs at the terminal level within the NAc (Phillips et al., 2003a), rather than indirectly through actions on VTA dopamine neurons. First, any decrease in the global activity of VTA dopamine neurons that is a consequence of BLA inactivation would result in decreased dopamine release following electrical stimulation of the VTA (Sombers et al., 2009). We clearly demonstrate that BLA inactivation has no effect on VTA-evoked
stimulated dopamine release in the NAc (Figure 2.7). Second, if BLA modulation of NAc dopamine was mediated via the VTA, we would expect a concurrent reduction in the probability of large concentration dopamine release events in response to the DS or during the baseline epoch following BLA inactivation, as dopamine transients of this magnitude (i.e. >40nM) reflect synchronous burst firing of VTA neurons (Sombers et al., 2009). However, BLA inactivation did not alter the Prob_{40} during either of these epochs. Together, these data indicate that BLA modulation of DS-related NAc dopamine release is not the result of alterations in burst firing of VTA dopamine neurons, but rather mediated, whether monosynaptically or polysynaptically, through terminal mechanisms within the NAc.

Perhaps the most intriguing aspect of these data lies in the functional consequence of BLA modulation of terminal NAc dopamine release. It is thought that the BLA is critical for maintaining the assigned value of conditioned stimuli, and using this information to guide subsequent instrumental responding (Cardinal et al., 2002). Specifically, disruption of BLA activity does not alter instrumental conditioning (Balleine et al., 2003) or simple Pavlovian autoshaping (Parkinson et al., 2000). However, BLA manipulation inhibits the formation of a conditioned place preference (Everitt et al., 1991; McDonald and White, 1993), Pavlovian-to-Instrumental Transfer (Corbit and Balleine, 2005), cue-induced reinstatement (Fuchs et al., 2006), second-order conditioning (Everitt et al., 1989; Setlow et al., 2002a), reward devaluation (Ostlund and Balleine, 2008; Johnson et al., 2009), and responding on high effort tasks (Simmons and Neill, 2009). Additionally, BLA inactivation during an effort-based task biases rats towards low-cost low-reward responding and decreased the willingness to expend higher effort for rewards suggesting that the BLA contributes to ascertaining the value of behavioral options (Ghods-Sharifi et al., 2009). Our data are consistent with those studies, as
a transient decrease of BLA activity decreased DS-evoked conditioned responses, but had no impact on instrumental responding. It is possible that a bilateral manipulation may have caused a significant disruption of instrumental responding, but our task required a relatively simple FR1 lever response, which is typically maintained following BLA manipulation (Whitelaw et al., 1996; Burns et al., 1999; Balleine et al., 2003). Further, phasic dopamine may contribute to switching attention to a salient stimulus (Pezze et al., 2007) since organisms must attend to a salient stimulus in order for it to guide subsequent behavior and the NAc may play a critical role in this process (Floresco et al., 2006).

At the cellular level, our findings indicate that glutamatergic inputs from the BLA to the NAc drive post-synaptic signals underlying reward-seeking (Ambroggi et al., 2008) and augment dopamine release at the terminal region. This concurrent activation may potentiate specific spatially- and temporally-linked synapses (Arbuthnott and Wickens, 2006) providing a mechanism by which the BLA can confer motivational value to environmental stimuli, and thereby play a role in modulating ongoing behavior. Numerous studies have demonstrated that dopamine-glutamate interactions are essential for neuroplasticity within reward circuits (Nicola et al., 2000), and interaction of BLA activity and NAc dopamine is necessary for conditioned responding to reward-predictive cues (Ambroggi et al., 2008). Further, the BLA-NAc circuit may prove to be critical in understanding dysfunction associated with drug addiction as recent evidence suggests that cocaine exposure leads to severe deficits in associative learning during reversal tasks (Jentsch et al., 2002; Schoenbaum et al., 2004; Calu et al., 2007), mediated by persistent miscoding of information within the BLA (Stalnaker et al., 2007). Our findings complement these results and provide a means for inflexible BLA
activation to induce inappropriate reward-seeking behavior governed by NAc output in the addicted state.
CHAPTER 3

BASOLATERAL AMYGDALA REGULATION OF NUCLEUS ACCUMBENS CELLULAR SIGNALING DURING CUED-INSTRUMENTAL RESPONDING FOR SUCROSE

ABSTRACT

The ability to process information regarding reward-predictive cues involves a diverse network of neural substrates. Given the importance of the nucleus accumbens (NAc) and the basolateral amygdala (BLA) in associative reward processes, recent research has examined the functional importance of BLA-NAc interactions. Here, multi-neuron extracellular recordings of NAc neurons coupled to microinfusion of GABA_A and GABA_B agonists into the BLA was employed to determine the functional contribution of the BLA to phasic neural activity across the NAc core and shell during a cued-instrumental task. Similar to previous reports, NAc neural response profiles prior to BLA inactivation exhibited largely indistinguishable activity across the core and shell. However, for NAc neurons that displayed increased firing rates relative to the cue during the task, BLA inactivation significantly reduced this activity selectively in the core (not shell). Additionally, phasic increases in firing rate in the core (not shell) immediately following the lever press response were also significantly reduced following BLA manipulation. Concurrent with these neural changes, BLA inactivation caused a significant increase in latency to respond and decrease in the percentage of trials in which animals made a conditioned approach to the cue. Together, these results suggest that an excitatory projection from the BLA provides a selective
contribution to conditioned neural excitations in NAc core neurons during a cued-instrumental task, providing insight into the underlying neural circuitry that mediates responding to reward-predictive cues.
INTRODUCTION

The nucleus accumbens (NAc) has long been described as an important neural substrate for reward processing, particularly in mediating the effects of motivationally relevant stimuli on goal-directed responding (Cardinal et al., 2002; Nicola, 2007; Humphries and Prescott, 2009). The NAc is a heterogenous structure, primarily comprised of two anatomically and functionally distinct subregions, the core and shell (Brog et al., 1993). Both of these structures are involved in appetitive reward behaviors, though numerous studies have attributed differential functional roles of the core and shell in conditioned reinforcement (Parkinson et al., 1999), action-outcome contingencies (Corbit et al., 2001b), and extinction and reinstatement (Fuchs et al., 2004). Correspondingly, NAc neuronal activity appears to track goal-directed responses (Peoples et al., 1997; Carelli, 2004; Nicola, 2007; Roesch et al., 2009) and cue-reward associations (Carelli, 2000; Setlow et al., 2003; Nicola et al., 2004b; Day et al., 2006; Hollander and Carelli, 2007). Despite clear dissociations in core and shell function, few differences in neural activity across subregions have been identified, specifically during reward behaviors (Ghitza et al., 2004; Hollander and Carelli, 2007). However, regional differences in NAc cell firing may be more readily apparent through circuit-level analysis (e.g., examination of the role of specific NAc afferents on NAc activity).

One critical input that has received increasing attention regarding its role in reward processing is the basolateral amygdala (BLA) (Everitt et al., 2000; Murray, 2007). Importantly, electrophysiological studies have revealed that BLA neurons are responsive to reward-predictive cues (Tye et al.; Carelli et al., 2003; Saddoris et al., 2005; Paton et al., 2006; Tye and Janak, 2007; Ambroggi et al., 2008). Recent hypotheses suggest that the BLA
is critical for maintaining the assigned value of conditioned stimuli, and using this information to guide subsequent behavior in the absence of rewards (Cardinal et al., 2002). Specifically, disruption of BLA activity does not alter instrumental conditioning (Balleine et al., 2003) or simple Pavlovian autoshaping (Parkinson et al., 2000). However, intact BLA function is necessary for the formation of a conditioned place preference (Everitt et al., 1991; McDonald and White, 1993), Pavlovian-to-Instrumental Transfer (Corbit and Balleine, 2005), cue-induced reinstatement (Fuchs et al., 2006), second-order conditioning (Everitt et al., 1989; Setlow et al., 2002a), reward devaluation (Ostlund and Balleine, 2008; Johnson et al., 2009), and responding on high effort tasks (Simmons and Neill, 2009).

Given the essential involvement of both the NAc and the BLA in incentive reward processes, a functional link of amygalo-striatal interactions has been proposed (Everitt et al., 1999). Neurophysiological evidence has shown that stimulation of BLA efferents evoke excitatory responses in NAc neurons (O'Donnell and Grace, 1995; Floresco et al., 2001c; Charara and Grace, 2003; Ambroggi et al., 2008; McGinty and Grace, 2008), and BLA activity can regulate phasic dopamine release within the NAc core (Jones et al., 2009). Indeed, the functional connectivity of the BLA-NAc pathway is necessary for animals to respond on a second-order conditioning task with a natural (Setlow et al., 2002b) or drug reinforcer (Di Ciano and Everitt, 2004). Additionally, recent evidence has shown that stimulus-controlled instrumental responding is also attenuated as a result of BLA-NAc disconnection (Ambroggi et al., 2008), as well as high effort fixed-ratio instrumental responding (Simmons and Neill, 2009).

Here, we used multi-unit extracellular electrophysiology of NAc activity coupled to microinfusion of GABA_A and GABA_B agonists (muscimol 0.03 nmol and baclofen 0.3 nmol
in 0.3µL) (McFarland and Kalivas, 2001) into the BLA, to determine the functional contribution of the BLA to phasic neural activity across the NAc core and shell during a cued-instrumental task. Similar to previous reports, NAc neural response profiles across the core and shell exhibited largely indistinguishable activity. However, distinct subsets of NAc neurons were differentially regulated by BLA activity, specifically subsets that correlated with presentation of conditioned cues. These results provide a critical characterization of afferent modulation by the BLA of phasic signaling in the NAc core and shell.
METHODS

Animals

Male Sprague Dawley rats (90–120 d old; Harlan, Indianapolis, IN) were used (n = 12). Rats had *ad libitum* access to water, with restricted food (Laboratory Rodent Diet; PMI Nutrition International, Branson, MO) limited to 15-25g per day to maintain weight between 85-95% of pre-surgical body weight. This regimen was in place for the duration of behavioral testing, except during the postoperative recovery period when food was given *ad libitum*. All procedures were approved by the University of North Carolina Institutional Animal Care and Use Committee.

Surgery

Animals were anesthetized with ketamine hydrochloride (100 mg/kg) and xylazine hydrochloride (20 mg/kg), and microelectrode arrays were implanted in the NAc using established procedures (Carelli et al., 2000). Electrodes were custom-designed and purchased from a commercial source (NB Laboratories, Dennison, TX, USA). Each array consisted of eight microwires (50 µm diameter) arranged in a 2x4 bundle that measured ~ 1.5 mm anteroposterior and ~ 0.75 mm mediolateral. Arrays were targeted for permanent, bilateral placement in the core and shell sub-regions of the NAc (AP, +1.3 to 1.7 mm relative to bregma; ML, ± 0.8 or 1.3 mm relative to bregma; DV, −6.2 mm from brain surface; (Paxinos and Watson, 2005)). Ground wires for each array were coiled around skull screws and placed into the ipsilateral side of the brain. Initially, rats (n=3) were implanted with unilateral guide cannula aimed at the BLA (anteriorposterior: -2.7 to -3.0; mediolateral: +/- 4.9 from bregma; dorsoventral: -6.2 from skull, (Paxinos and Watson, 2005)). To increase
the yield of data from each animal, bilateral guide cannula were implanted in the remaining rats (n=9). BLA guide cannula consisted of a 22 gauge thin-walled stainless-steel tubing (Plastics One Inc., Roanoke, VA) and were lowered to 2 mm above the target site. The cannula and microarrays were secured with stainless steel screws and dental acrylic. Stylets (Plastics One Inc., Roanoke, VA) were inserted into the length of the guide cannula to maintain patency.

Electrophysiological recordings

Electrophysiology procedures have been described previously (Carelli et al., 2000). Briefly, before the start of each session, subjects were connected to a flexible recording cable attached to a commutator that allowed virtually unrestrained movement within the chamber. NAc activity was recorded differentially between the active and the inactive electrodes from the permanently implanted microwires. On-line isolation and discrimination of neuronal activity was accomplished using a neurophysiological system commercially available (MAP system; SIG board filtering, 250 Hz to 8 kHz; sampling rate, 40 kHz; Plexon, Dallas, TX). Individual waveforms corresponding to a single cell were discriminated using template analysis procedures provided by the MAP system and sorted further after each experiment using principal component analysis in Offline Sorter (Plexon, Dallas, TX). Raster displays and perievent histograms (PEHs) were constructed using commercially available software (NeuroExplorer; Plexon, Dallas, TX).

Apparatus
Experimental sessions occurred in $43 \times 43 \times 53$ cm Plexiglas chambers (Medical Associates, St. Albans, VT, USA) housed within sound-attenuating boxes (Medical Associates, St. Albans, VT, USA). Two symmetrically located retractable levers (Colburn Instruments, Allentown, PA, USA) were placed 17 cm apart on one wall of the chamber. Cue lights were positioned above each lever. A food receptacle was centered between the levers, 2.5 cm from the floor. A house light was centrally located on the wall opposite the food receptacle and levers, 2 cm from the ceiling.

**Behavioral Task**

Rats were trained to respond for sucrose (45 mg pellets; Test Diet) delivered into a receptacle on a discriminative stimulus fixed-ratio 1 (FR1) schedule of reinforcement. Sessions were limited to a specified number of pellet deliveries (90 pellets/day) in a single daily session. Each training session was initiated by the onset of the houselight, presentation of continuous white noise, and insertion of the inactive lever. Responses on the inactive lever had no programmed consequences. During the operant training session, either the discriminative stimulus (DS) or non-associated stimulus (NS) was presented on a variable-interval schedule with an average inter-trial interval of 20s. Figure 1a presents a schematic of the behavioral task. The DS and NS were semi-randomly presented over three trials with a 2:1 ratio of DS:NS frequency. The DS consisted of a unique audio tone (either 1000 or 2750 Hz; counterbalanced across animals) presented for 6s. Upon termination of the tone, the active lever was inserted into the chamber and the associated cue-light was illuminated. An instrumental response on the active lever resulted in lever retraction, cue-light termination and delivery of sucrose into the receptacle, as well as an additional unique 6s audio tone
If the rat did not respond on the active lever within 30s of presentation, the cue-light was terminated and the lever was retracted, and a new inter-trial interval was initiated. The NS consisted of the alternative tone (1000 or 2750 Hz) and was also presented for 6s. Following NS offset, there were no additional programmed consequences. Rats underwent 10-13 days of training until criterion was met with accurate responses made on greater than 95% of DS trials across 3 consecutive sessions.

![Schematic of the operant task](image)

**Figure 3.1.** Schematic of the operant task. During sessions, one of two trials was presented, the DS or NS. The DS was presented for 6s, after which the active lever (L\textsuperscript{a}) was extended. Responses on the L\textsuperscript{a} resulted in sucrose delivery and CS onset (6s). Responses during the NS resulted in no programmed consequences. The inactive lever (L\textsuperscript{i}) was always present. Black triangles denote a lever response.

*Experimental test day and microinfusion*

Once trained, all rats underwent surgical procedures described above. Following recovery, animals had 5 additional operant training days. On the final two re-training days, rats were tethered and the session was divided into pre and post segments to acclimate each animal to the test day procedure, and mock microinfusions were given. On the experimental test day, NAc cell firing was recorded during a session that consisted of 135 total trials (90 DS and 45 NS; described above), divided into the pre-infusion (PRE) and post-infusion (POST) periods. After the initial 45 pre-infusion trials rats were given a 0.3µL unilateral microinfusion of either vehicle (0.9% NaCl sterile saline; VEH) or drug (0.3nmol baclofen
GABA_B agonist/0.03nmol muscimol GABA_A agonist, in 0.9% NaCl sterile saline; BM; dose from (McFarland and Kalivas, 2001)) into one BLA cannula. Microinfusions were made over 1 min, and the injector remained in place for a 1 min post-injection diffusion period. All 12 rats received at least 2 test days, with unilateral injection of either VEH or BM. The 9 rats with bilateral cannula also received 2 additional test days in the alternate unilateral cannula. In all cases, the order of treatment was randomly assigned for each animal (alternating infusion side between days). After the microinfusion, the animals were immediately reconnected to the recording apparatus and placed in the chamber, and post-infusion trials commenced 5 min later.

Behavioral analysis

Several behavioral measures were examined: the DS and NS approach response ratios, the DS response latency, and the number of overall responses on the active and inactive levers. Approach responses were analyzed through video analyses, wherein an approach was defined as a directed movement or orienting of the animals head into the active lever region of the chamber (2in. x 2in. around the lever), during the DS or NS presentation. Response latency and lever presses were recorded via computer.

Neural analysis

NAc neuronal firing patterns were characterized using raster displays and peri-event histograms (PEHs) constructed with commercially available software (NeuroExplorer, Littleton, MA, Plexon, Dallas, TX). Raster displays and PEHs display the activity of each cell time-locked to particular behavioral events. For each cell, PEHs were constructed time-
locked to the DS, NS or operant response. To examine neural activity relative to DS presentation, cell firing was examined during 0-1s following DS onset compared to the ‘DS baseline' period, (−5 to 0 s prior to DS presentation). To determine neural activity relative to NS presentation cell firing was examined during the 0-1s following NS onset compared to the ’NS baseline' period (−5 to 0 s prior to NS presentation). To evaluate cell firing before and following the lever press response, cell firing was examined during the 1s period preceding the operant response (-1 – 0 pre-response) and 1s following the response (0 – 1 post-response) compared to baseline activity (the DS baseline period was used since no events occurred during that time). Next, 99.9% confidence intervals (CI) were projected in each PEH from the baseline epochs for each neuron (Neuroexplorer). Neural responses were characterized as phasic by the presence of at least one bin (beginning within each event epoch) that showed an increase or decrease in firing rate over the projected baseline CI. A subset of cells exhibited larger variability in their baseline firing rates and the projected 99.9% CI included zero. For these neurons to be classified as phasic, \( e_0 > 2 b_0 \) had to be true (where \( e_0 \) = consecutive zero bins occurring within the event epoch, and \( b_0 \) = maximal number of consecutive zero bins in the baseline epoch).

Next, each NAc neuron was classified into specific response profiles (types), as follows. Cells that increased their firing rate in the 1s following cue-onset were classified as DSe or NSe, according to the respective cue. For cells that increased their firing rate surrounding the operant response, if the increase was marked as phasic in the pre-response period, it was considered a pre-response excitation (PRe), even if this phasic increase persisted into the post-response period. Cells that increased their firing rate only in the post-response period were classified as reinforcement excitation (RFr) neurons. Note, PRe and
RFe are inherently mutually exclusive, but both types of operant responses could also be classified as cue-responsive (i.e. DSe). NAc neurons that decreased their firing rate in the 1s following cue-onset were classified as DSi or NSi respectively. Cells that decreased their firing rate surrounding the operant response were classified as operant inhibitions (OPi). Pre-response and post-response inhibitions were collapsed as the incidence of responses in only one period was extremely small. Again, OPi could also be classified as cue-responsive (e.g., DSe).

Two exclusion criteria were used to remove neurons that did not meet inclusion standards noted above. Cells were excluded if the baseline firing rate for the PRE infusion period was less than 0.2 Hz or if the cell had fewer than 500 spikes within this period. Cells were also removed from analysis if the baseline firing rate during the PR period exceeded 10 Hz, as these were likely not medium-spiny neurons (Berke et al., 2004). Using this approach, of the 303 units recorded across all sessions, 15 were excluded based upon the first, and 40 units were excluded based on the second criteria.

Histology

Rats were deeply anesthetized with a ketamine and xylazine mixture (100 mg/kg and 20 mg/kg, respectively) and perfused transcardially using physiological saline, 10% formalin and 3% potassium ferricyanide, and brains were removed. In order to mark the placement of electrode tips, a 13.5 µA current was passed through each microwire electrode for 5 s. To mark microinfusion sites, a brief 5 µA current was passed through injector needles placed into the guide cannula. After post-fixing and freezing, 40-µm coronal brain sections were
mounted and stained with thionin. Electrode tip locations and injection sites were identified based upon anatomical organization (Paxinos and Watson, 2005).

Statistics

Analysis of behavioral data was completed using paired t-tests. To examine acquisition of the task, a two-way repeated measures ANOVAs involving session number x cue (DS v NS) was employed. To determine the effects of BLA inactivation on cue-evoked approach behavior a two-way repeated measures ANOVA of cue (DS v NS) x treatment (VEH v BM) was employed. All ANOVAs were followed by Bonferroni post hoc tests as noted. Proportions and frequencies of units and wire placements across subregions were compared using chi-square tests and Fisher’s exact test (GraphPad Prism 4).

To compare differences in mean firing rate of individual response patterns across subregions, a two-way repeated measures ANOVA of epoch (baseline v signal) x subregion (core v shell) was used. To determine the effects of BLA manipulation on cell firing, PEH were constructed for each individual neuron across the PRE and POST infusion periods to compare the within-session changes in firing. Cells were grouped based on their hemispheric relation to the infusion side as well as by subregion. To examine changes within each cell group, a three-way repeated measures ANOVA of epoch (baseline v signal) x infusion (PRE v POST) x treatment (VEH v BM) was used. The treatment variable acted as the between-subjects factor. Statistics were analyzed using either Prism 4 (GraphPad) or SPSS 17.0 (SPSS).
RESULTS

Histology

Histological verification of electrode placement across NAc sub-regions revealed that there was no significant difference in the distribution of wires between the core (n=76) and shell (n=94) ($\chi^2 = 1.91; \ p=0.17$) (Figure 3.2). Likewise, sixteen micro-infusion sites were histologically confirmed to be in the BLA. Non-BLA infusion sites (n=5) and non-NAc wire placements were excluded from analysis.

![Figure 3.2. Histological representation of electrode and cannula placement. Left diagrams show the NAc placements of electrodes at recording. Right diagrams illustrate the tip of the microinjector placed into the BLA cannula (black circles) and misses within the piriform cortex (tan circles) (Paxinos, 2005)](image)

Behavior

Animals learned to successfully discriminate between the active and inactive levers and reached DS responding criterion within 13 sessions. A two-way ANOVA (lever x session) revealed a significant interaction ($F_{(1,12)} = 27.45, \ p < 0.0001$) across the final 8
training sessions and subsequent retraining days (Figure 3.3a). Bonferroni post hoc tests revealed that over the final 6 training sessions and all retraining days there were significantly more responses on the active than the inactive lever (p<0.001).

Unilateral inactivation of the BLA had no significant effect on the ability of the rats to perform the behavioral task (t_{11}=1.44, \ p=0.17; Figure 3.3b). To examine the effect of BLA inactivation on conditioned responses to DS or NS presentation, both the latency to respond and the percentage of trials in which animals approached the active lever during cue presentation was determined. Compared to VEH, BM infusion caused a significant increase in the latency to respond (t_{11}=2.718, \ p=0.02; Figure 3.3c). Further, a two-way repeated measures ANOVA revealed significant main effects of treatment (VEH and BM, F_{(1,22)} = 26.67, \ p<0.0001) and cue (DS and NS, F_{(1,22)} = 201.7, \ p<0.0001) and a significant interaction (treatment x cue; F_{(1,22)} = 12.89, \ p=0.0016) on the percentage of trials in which animals approached the active lever during cue presentation (Figure 3.3d). Specifically, BM treatment significantly decreased the percentage of DS trials in which the animals made an approach (p < 0.01). There was no significant difference between the VEH or BM treatment in NS trial approaches (p>0.05). Together, these data demonstrate that unilateral inactivation of the BLA attenuates DS-related cue-evoked behavior.
Figure 3.3. Behavioral responding following BLA treatment. a) Average number of lever presses across the final eight training sessions and subsequent retraining days. b) Average percentage of DS trials with an active lever response following BLA treatment. c) The average change in DS response latency following BLA treatment. d) The average percentage of trials with a cue-evoked approach (DS or NS) following BLA treatment. Data and error bars reflect mean ± SEM. ns denotes p>0.05; * denotes p<0.05.

Subregion characterization of NAc neural activity

To characterize NAc cell firing during the task, the neural response profiles of neurons collected from the PRE period on the initial VEH day for each animal were analyzed. A total of 126 NAc neurons were recorded from 12 rats. There was an even distribution in cell numbers across the NAc core (n=66) and shell (n=60) (Fisher’s Exact Test p=0.19). Moreover, there was no significant difference in the baseline (−5 to 0 s prior to DS onset) firing rate of neurons in the core (2.12 ±0.24 spikes/s) versus the shell (2.34±0.27 spikes/s; t_{(124)}=0.61, p=0.54).

Table 3.1 shows the number of neurons that exhibited a change in firing rate during the four behavioral epochs (DS, NS, Pre-response, Post-response) analyzed. It is important
to note that these groups are not mutually exclusive, as neurons often demonstrated multiple
response patterns. As is evident in Table 3.1, the majority of NAc neurons exhibited phasic
activity during the task. Moreover, a greater proportion of NAc core neurons exhibited at
least one phasic response (57/66; 86.4%) compared to neurons in the NAc shell (42/60;
70.0%) (Fisher’s exact test; p=0.03).

<table>
<thead>
<tr>
<th>Neural response</th>
<th>Analysis window</th>
<th>Core n=66</th>
<th>Shell n=60</th>
</tr>
</thead>
<tbody>
<tr>
<td>DS</td>
<td>e 0-1s post DS</td>
<td>12 18.2%</td>
<td>7 11.7%</td>
</tr>
<tr>
<td></td>
<td>i 0-1s post DS</td>
<td>13 19.7%</td>
<td>11 18.3%</td>
</tr>
<tr>
<td>NS</td>
<td>e 0-1s post NS</td>
<td>9 13.6%</td>
<td>4 6.7%</td>
</tr>
<tr>
<td></td>
<td>i 0-1s post NS</td>
<td>7 9.1%</td>
<td>6 10.0%</td>
</tr>
<tr>
<td>Pre-response</td>
<td>e 0-1s before lever press</td>
<td>18 27.3%</td>
<td>8 13.3%</td>
</tr>
<tr>
<td></td>
<td>i 0-1s before lever press</td>
<td>17 25.8%</td>
<td>18 30.0%</td>
</tr>
<tr>
<td>Post-response</td>
<td>e 0-1s after lever press</td>
<td>28 42.4%</td>
<td>21 35.0%</td>
</tr>
<tr>
<td></td>
<td>i 0-1s after lever press</td>
<td>24 36.4%</td>
<td>19 31.7%</td>
</tr>
</tbody>
</table>

Table 3.1. These data were taken from the first VEH recording session from each animal. The percentages within the table are based on the number of neurons recorded from each subregion (n=66 core; n=60 shell; n=126 total). Of the 126 neurons, 108 exhibited phasic activity (i.e. at least one of the given response profiles).

Cells were then assigned to specific types and further analyzed. Examples of
individual NAc neurons that exhibited excitatory response profiles are shown in Figure 3.4.
An example of a neuron that exhibited type DSe activity, characterized by significant
increases in firing rate during the 1s following cue-onset, is shown in Figure 3.4a (increased
activity highlighted in gray bar). There was no significant difference in the proportion of
neurons that exhibited DSe response profiles across the core (n=12) and shell (n=7) (Fisher’s
exact test; p=0.33). Although DSe neurons in each subregion showed significant increases in
firing rate during the signal period (i.e., 1 s following cue onset) compared to baseline
(Figure 3.4b) a two-way repeated measures ANOVA (subregion x epoch) was used to
determine whether there were differences in DSe response profiles across subregions. The ANOVA revealed a significant main effect of epoch ($F_{(1,17)} = 8.97$, $p<0.01$), but not subregion ($F_{(1,17)} = 1.61$, $p=0.22$), indicating the magnitude of the DSe firing rates and baselines were similar between the core and shell.

Figure 3.4. A subset of NAc neurons in the core and shell exhibit phasic excitations in cell firing relative to cue onset or the lever press response. a) PEH and raster display show the activity of a representative DSe neuron; activity is aligned to DS-onset (left panel; dotted line) or the lever press response (right panel; dotted lined). DSe epoch (signal period) is denoted by the gray bar. b) Average firing rate across baseline and signal periods for all DSe neurons in the core and shell. c) PEH and raster display show the activity of representative PRe neuron; activity aligned to DS-onset (left panel; dotted line) or the lever press (right panel; dotted lined). PRe epoch (signal period) is denoted by the gray bar. d) Average firing rate during the baseline and signal periods for all PRe neurons in the core and shell. e) PEH and raster display show activity of a representative RFe neuron; firing aligned to DS-onset (left panel; dotted line) or operant response (right panel; dotted lined). RFe epoch (signal period) is denoted by the gray bar. f) Average firing rate during the baseline and signal periods for all RFe neurons in the core and shell. Data and error bars reflect mean ± SEM. * denotes $p<0.01$. 
PRe neurons were characterized by significant increases in firing rate during the 1s before the operant (lever press) response. The PEH in Figure 3.4c shows the activity of a representative PRe neuron. The proportion of neurons that exhibited PRe response profiles were not statistically different across the core (n=18) and shell (n=8), although this did approach significance (Fisher’s exact test; p=0.08). Likewise, PRe neurons in each subregion showed significant increases in firing rate during the signal period (i.e., 1 s preceding operant response) compared to baseline (Figure 3.4d). Additionally, the magnitude of the PRe signal and the baseline firing rate was similar across the core and shell, as a two-way repeated measures ANOVA (subregion x epoch) revealed a significant main effect of epoch (F(1,24) = 14.69, p<0.001), but not subregion (F(1,24) = 0.82, p=0.38).

RFe neurons were characterized by significant increases in firing rate during the 1s period signal following the response. Neurons that exhibited RFe response profiles (example shown in Figure 3.4e) were also similarly distributed across the core (n=15) and shell (n=15) (Fisher’s exact test; p=0.84). RFe neurons showed significant increases in firing rate during the signal period compared to baseline (Figure 3.4f). The magnitude and baseline firing rate of RFe neurons was similar across the core and shell, as a two-way repeated measures ANOVA (subregion x epoch) revealed a significant main effect of epoch (F(1,28) = 54.14, p<0.0001), but not subregion (F(1,28) = 1.61, p=0.22).

Neurons that exhibited inhibitory response profiles relative to behavioral events were classified as follows. DSi response neurons were characterized by significant decreases in firing rate during the 1s following DS onset. An example of a representative DSi neuron is shown in Figure 3.5a (gray bar), and the decline in firing rate during the signal relative to baseline periods for all DSi cells in the core and shell is shown in Figure 3b. There was no
significant difference in the proportion of NAc neurons that were classified as DSi across the core (n=13) and the shell (n=11) (Fisher’s exact test; p=1.0). Likewise the magnitude of the inhibition for DSi cells was similar across subregions as the two-way repeated measures ANOVA (subregion x epoch) revealed a significant main effect of epoch ($F_{(1,22)} = 15.29$, p<0.001), but not subregion ($F_{(1,22)} = 1.40$, p=0.25) (Figure 3.5b). OPi neurons were characterized by significant decreases in firing rate during the 2s surrounding the operant response. Figure 3.5c shows an example of a representative OPi neuron (Figure 3.5c gray bar) and the mean firing rates during the signal and baseline periods across all OPi cells in the core and shell (Figure 3.5d). OPi response profiles were also evenly distributed across the core (n=31) and shell (n=23) (Fisher’s exact test; p=0.37). Likewise, the OPi baseline and epoch signal were similar between subregions as the two-way repeated measures ANOVA (subregion x epoch) revealed a significant main effect of epoch ($F_{(1,52)} = 44.74$, p<0.0001), but not subregion ($F_{(1,52)} = 0.80$, p=0.37) (Figure 3.5d). Taken together, these results demonstrate a remarkable similarity in the neural response profiles of NAc neurons across the core and shell during the task.
Figure 3.5. Another subset of NAc neurons (types DSi and OPi) show phasic inhibitions in cell firing relative to cue onset or the lever press response in the core and shell. a) PEH and raster display of a representative DSi neuron aligned to DS-onset (left panel; dotted line) or the lever press (right panel; dotted lined). DSi epoch (signal period) is denoted by the gray bar. b) Average baseline and signal firing rates for all DSi neurons in the core and shell. c) PEH and raster display showing the activity of a representative OPi neuron aligned to DS-onset (left panel; dotted line) or operant response (right panel; dotted lined). OPi epoch (signal) is denoted by the gray bar. d) Average baseline and signal firing rate for all OPi neurons in the core and shell. Data and error bars reflect mean ± SEM. * denotes p<0.01.

**BLA regulation of NAc phasic excitations**

The primary goal of this study was to determine the contribution of BLA activity to phasic neural responses within the NAc. As noted above, the pharmacological manipulation used in this study induced a significant reduction in conditioned responses following the presentation of the DS, with no significant disruption in instrumental responding. As such, it was hypothesized that BLA inactivation would attenuate neural firing within NAc phasic excitations, specifically DS-evoked signals.

First, it was determined whether DSe responses were altered as a function of BLA inactivation. To this end, the firing rates of DSe neurons were compared before and following BLA inactivation. An example DSe core neuron before and after BM is shown in Figure 3.6a. For the population, a three-way repeated measures ANOVA (epoch x infusion x treatment) revealed a significant interaction of epoch x infusion x treatment ($F_{(1,11)} = 7.26$, $p=0.02$). This finding reveals that ipsilateral DSe neurons in the NAc core exhibited a significant reduction in the DS-evoked signal as a result of BM treatment (Figure 3.6b). However, there was no significant attenuation of the DSe signal in the NAc shell following BLA inactivation, with only a main effect of epoch ($F_{(1,6)} = 34.10$, $p=0.001$) and no significant interaction of epoch x infusion x treatment ($F_{(1,6)} = 0.80$, $p>0.05$) (Figure 3.6c). Importantly, this attenuation of DSe signal was specific to neurons ipsilateral to the BLA.
treatment, as neither neurons in the core (epoch x infusion x treatment; F(1,2) = 3.65, p >0.05) or shell (epoch x infusion x treatment; F(1,9) = 1.62, p >0.05) exhibited a significant change in signal when the contralateral BLA was inactivated.

Next, the effect of BLA inactivation on PRe response profiles was examined. A representative PRe neuron from the NAc core is shown in Figure 3.6d. For PRe neurons in both the core (Figure 3.6e) and shell (Figure 3.6f), there was no significant change in signal compared to baseline firing as a function of BLA manipulation (core; epoch x infusion x treatment F(1,11) = 1.33, p>0.05; shell; epoch x infusion x treatment F(1,6) = 0.23, p>0.05). Furthermore, there was no significant change in PRe signal in neurons contralateral to the BLA manipulation in either the core or shell (core; epoch x infusion x treatment F(1,9) = 4.91, p>0.05; shell; epoch x infusion x treatment F(1,9) = 0.59, p>0.05).

Third, the effects of BLA inactivation on RFe response profiles were examined. A representative RFe neuron from the NAc core is shown in Figure 3.6g. For RFe neurons in the core, a significant reduction in the DS-evoked signal was observed following BLA inactivation (Figure 3.6h). However, this reduction in type RFe activity was not specific to BM treatment as there was not a significant three-way interaction (epoch x infusion x treatment F(1,11) = 3.12, p>0.05), but a significant epoch x infusion interaction (F(1,11) = 15.49, p=0.002). That is, the reduction in RFe activity was observed following both BM and VEH infusion into the BLA. However, the average reduction of the RFe signal was larger in the BM (64.2%) versus VEH (18.9%). Interestingly, again neurons in the shell exhibited no significant change in signal as a result of BLA manipulation (epoch x infusion x treatment F(1,8) = 1.14, p>0.05), but only a main effect of epoch (F(1,8) = 16.00, p=0.004) (Figure 3.6i). This selective attenuation of RFe signal in the core, but not the shell, is also specific to
ipsilateral neurons, as there was no significant change in contralateral RFe signals in either
the core or shell (core; epoch x infusion x treatment $F_{(1,8)} = 1.37$, $p>0.05$; shell; epoch x
infusion x treatment $F_{(1,11)} = 3.56$, $p>0.05$).

Figure 3.6. Effects of BLA inactivation on NAc neurons exhibiting phasic excitations (types
DSe, PRe and RFe). a) PEH and raster displays show the activity of a representative DSe
neuron in the NAc core before (black) and following (blue) BM treatment aligned to DS-
onset (left panel; dotted line) and the lever press response (right panel; dotted lined).
Average change (defined as POST firing rate/PRE firing rate) in baseline and signal firing
rates for all DSe neurons in the core (b) and shell (c) following vehicle (VEH) or BM
treatment. d) PEH and raster display show the activity of a representative PRe neuron in the
core before (black) and following (blue) BM treatment aligned to cue-onset (left panel; dotted line) and the lever response (right panel; dotted lined). Average change in baseline
and signal firing rates for all PRe neurons in the core (e) and shell (f) following VEH or BM
treatment. g) PEH and raster showing example RFe core neuron before (black) and
following (blue) BM treatment aligned to cue-onset (left panel; dotted line) and lever
response (right panel; dotted lined). Average change baseline and signal firing rates for all in
RFe cells in the core (h) and shell (i) following VEH or BM treatment. Data and error bars
reflect mean ± SEM. * denotes $p<0.05$. 
**BLA regulation of NAc phasic inhibitions**

For DSi neurons across both the core and shell, there was no significant change in DSi signal compared to baseline firing as a function of BLA manipulation (core; epoch x infusion x treatment $F_{(1,11)} = 0.54$, $p>0.05$; shell; epoch x infusion x treatment $F_{(1,12)} = 0.25$, $p>0.05$) (Figure 3.7a and b). Furthermore, there was no significant change in contralateral DSi signal compared to baseline firing as a result of BLA manipulation in either the core or shell (core; epoch x infusion x treatment $F_{(1,11)} = 0.54$, $p>0.05$; shell; epoch x infusion x treatment $F_{(1,12)} = 0.25$, $p>0.05$).

The effects of BLA inactivation on OPi cell firing across the core and shell was also examined. If a neuron was not phasic across the entire OPi period, only the period in which it was phasic (i.e. preOP or postOP) was evaluated. For OPi neurons in the core, there was no significant change in OPi signal as a function of BLA manipulation (epoch x infusion x treatment $F_{(1,29)} = 1.13$, $p>0.05$) (Figure 3.7c). However, OPi neurons in the shell exhibited a significant change in the baseline firing rate across both BLA treatments, with a significant epoch x infusion interaction ($F_{(1,26)} = 10.06$, $p=0.004$) (Figure 3.7d). Interestingly, the change in baseline firing rate was similar across VEH (78.1% of PRE) and BM (73.1% of PRE) treatments. Furthermore, there was no significant change in the contralateral DSi signals across either the core or shell (core; epoch x infusion x treatment $F_{(1,19)} = 0.35$, $p>0.05$; shell; epoch x infusion x treatment $F_{(1,21)} = 2.16$, $p>0.05$). The functional significance of this reduction in inhibitory responding is unclear, but the finding that it occurred under both VEH and BM conditions indicates that any unknown behavioral consequences of this neural profile are not uniquely dependent upon BLA activity.
Figure 3.7. Effects of BLA inactivation on NAc neurons exhibiting phasic inhibitions (types DSi and OPi). Average change in baseline and signal firing rates across all DSi neurons in the core (a) and shell (b). Average change in baseline and signal firing rates across all OPi neurons in the core (c) and shell (d). Data and error bars reflect mean ± SEM. * denotes p<0.05.

Experimental controls

To demonstrate that the results presented are not the result of diffusion of BM from the BLA to neighboring regions, we examined infusion sites located outside the BLA. These sites were located within regions of the piriform cortex, lateral to the target. Figure 3.8 illustrates the effects of BM treatment on the behavioral responding following BLA infusions (n=3). Paired t-tests comparing the effect of BLA treatment on DS-response latency revealed no significant difference between VEH or BM treatments ($t_{(2)}=0.80$, $p=0.50$; Figure 3.8a). Further, a two-way repeated measures ANOVA comparing the proportion of trials with a DS-evoked approach revealed no significant main effects of treatment ($F_{(1,4)} = 0.08$, $p>0.05$), infusion ($F_{(1,4)} = 7.2$, $p>0.05$) or an interaction ($F_{(1,4)} = 3.2$, $p>0.05$) (Figure 3.8b). Figure 3.8c shows the DSe responsive core neuron located ipsilaterally to the piriform treatment.
before and following BM treatment, illustrating no significant reduction in the DSe magnitude.

Figure 3.8. Effects of inactivation of piriform locations (n=3). (a) The mean change in DS response latency (post-infusion latency / pre-infusion latency) across VEH and BM treatments. (b) The average percentage of DS trials with a cue-evoked approach across both VEH and BM treatments. (c) A representative NAc core neuron exhibiting a DSe response (DS-onset dotted line) ipsilateral to the piriform BM treatment. Data and error bars reflect mean ± SEM. ns denotes p>0.05.
DISCUSSION

The present findings demonstrate that the BLA differentially regulates conditioned neural responses within the NAc core and shell. Specifically, DS-evoked excitations for neurons in the NAc core, but not the shell, exhibited a significant reduction in firing rate following BM inactivation of the BLA. Additionally, excitations in firing rate in the core (not shell) immediately following the lever press response were also significantly reduced following BLA manipulation. Concurrent with these neural changes, BLA inactivation caused a significant increase in latency to respond and decrease in the percentage of DS trials in which animals made an approach response. Together, these results suggest that an excitatory projection from the BLA provides a selective contribution to conditioned neural excitations in NAc core neurons during a cued-instrumental task, providing insight into the underlying neural circuitry that mediates responding to reward-predictive cues.

The primary finding reported here is that distinct subsets of NAc core neurons, but not shell neurons, are regulated by BLA activity during the task. Recent evidence demonstrated that BLA activity facilitates incentive cue responses of NAc core neurons, concomitant with a decrease in stimulus-controlled behavior (Ambroggi et al., 2008). However, that study was restricted to neural activity within the NAc core. Here, both core and shell neural responses were examined. Despite similarities in neural response profiles between these subregions prior to pharmacological manipulation of the BLA, BLA inactivation differentially mediates DS-evoked excitations in the NAc core. As noted above, we also examined neural activity surrounding the operant response, and show that post-response excitations within the core were also attenuated by BLA inactivation. Such post-response excitations have typically been associated with encoding of the CS (i.e., a distinct
cue paired with reward delivery) (Carelli and Deadwyler, 1997; Carelli, 2000). We did not explicitly test this possibility with non-contingent CS probes, but it is likely that these responses encode aspects of the conditioned stimulus (Carelli and Deadwyler, 1997), the consummatory response (Nicola et al., 2004a) or reinforcer palatability (Taha and Fields, 2005). Given that the NAc core has been shown to exhibit more phasic excitations in response to a CS that evokes conditioned approaches (Day et al., 2006), it is likely that the attenuated post-response signal found here is a similar subset of CS-responsive core neurons. Taken together, these data suggest that BLA activity drives conditioned phasic excitations within the core, but not the shell, specifically via an ipsilateral connection.

These results are consistent with anatomy studies which show that the BLA sends primarily glutamatergic efferents to the NAc (Kelley et al., 1982; McDonald, 1991; Brog et al., 1993). Previous electrophysiological studies have shown that stimulation of BLA efferents evoke excitatory responses in NAc neurons (O'Donnell and Grace, 1995; Floresco et al., 2001c; Charara and Grace, 2003; Ambroggi et al., 2008; McGinty and Grace, 2008). However, the differentiation between core and shell neural responses in our study is somewhat surprising, as the BLA projects to both the core and shell, with a great deal of overlap in projections within our BLA region of manipulation (Shinonaga et al., 1994). As such, these data provide evidence that contributions of the BLA to NAc core signaling may reflect the behavioral specificity required during our cued-instrumental task. Thus, the differential effects of BLA inactivation on NAc cell firing in the core and shell revealed here is consistent with numerous studies that demonstrate functional dissociations between these NAc sub-regions. As such, our finding may reveal a novel neural correlate in the core that is critical in processing discrete reward-predictive stimuli used to guide appetitive responding.
Specifically, transient inactivation of the NAc core attenuates the expression of conditioned approach responses to a CS (Blaiss and Janak, 2009), instrumental conditioned reinforcement (Di Ciano et al., 2008), and cue-induced reinstatement (Fuchs et al., 2004; Floresco et al., 2008). Conversely, the shell appears to have a greater role in suppressing competing responses, independent of discrete cue presentation, as transient inactivation of the shell decreases conditioned approach to a reward-predictive CS (Blaiss and Janak, 2009) or DS (Ambroggi et al., 2009), but also dramatically increases non-specific approach behavior in response to a non-associated cue (Ambroggi et al., 2009; Blaiss and Janak, 2009). Furthermore, in a cue-reinstatement paradigm, inactivation of the shell can actually enhance cue-induced reinstatement responding indiscriminately across operands (Floresco et al., 2008). Furthermore, the reported differential contribution of BLA activity to the NAc core is consistent with a growing literature on the amygalo-striatal network (Cardinal et al., 2002). Specifically, BLA-NAc core interactions have been shown to be critically important to reward processes such as conditioned place preference (Everitt et al., 1991), second-order conditioning (Setlow et al., 2002b; Di Ciano and Everitt, 2004) and stimulus-controlled instrumental responding (Ambroggi et al., 2008).

Although the present findings illustrate the importance of the BLA-NAc core projection in driving cue-evoked responding, there are additional circuits and mechanisms to consider. For example, studies have demonstrated that ventral tegmental area (VTA) inactivation reduces behavioral responding, as well as NAc excitations and inhibitions, evoked by a DS (Yun et al., 2004b). Likewise, predictive cues elicit robust phasic dopamine release in the NAc core (Day et al., 2007), and behavioral responding to cues is dopamine dependent (Nicola et al., 2005). Moreover, VTA inactivation also produces a robust decrease
in baseline firing rate in NAc neurons, suggesting that tonic firing may be permissive of cue-evoked responses (Yun et al., 2004b). In the present study, however, BLA inactivation had no effects on baseline firing rates of NAc core neurons, while significantly reducing DS-evoked excitations. Coupled with the increased latency to behaviorally respond the DS, our results argue that phasic, cue-evoked excitation of NAc core neurons may play a role in driving the behavioral response to cues. This interpretation is further supported by evidence that the magnitude of the phasic DS-evoked excitations in the NAc core predict whether a subsequent behavioral response to the cue is made (Nicola et al., 2004b).

If cue-evoked excitation of NAc core neurons drives cue-evoked responding, it is possible that these responses are also influenced by regions of the prefrontal cortex (PFC). Similar to the BLA, there is a direct, predominantly ipsilateral excitatory projection from the PFC to the NAc core (Sesack et al., 1989; Brog et al., 1993). Neurons in the NAc receive convergent input from the mPFC and the VTA (Sesack and Pickel, 1992). Activity within the PFC is essential for responding to a reward-predictive cue (Ishikawa et al., 2008a), and inactivation of the dorsomedial PFC reduces DS-excitations, as well as inhibitions, in NAc neurons (Ishikawa et al., 2008b). However, large proportions of NAc neurons that are activated by the PFC are also activated by the BLA (O'Donnell and Grace, 1995), and BLA input can directly gate (Goto and O'Donnell, 2002) or indirectly modulate PFC to NAc excitations (McGinty and Grace, 2008). Thus, it is likely that convergent excitatory inputs from both the BLA and mPFC, and dopaminergic input from the VTA, are required for activation of NAc core neurons by reward-predictive cues.

Therefore, we propose that a complex neural circuit, wherein converging phasic information from the BLA, PFC and VTA are integrated within the NAc core to influence
cue-evoked responding. Phasic dopamine release within the NAc in response to reward-predictive cues is terminally regulated by the BLA (Jones et al., 2009), likely via actions on pre-synaptic glutamate receptors (Floresco et al., 1998; Phillips et al., 2003a). Furthermore, concomitant activation of D1 and NMDA receptors in the NAc core is necessary for appetitive instrumental learning (Smith-Roe and Kelley, 2000) and D1 and NMDA receptor activation is necessary for BLA-induced potentiation of NAc signaling (Floresco et al., 2001c). Additionally, recent studies suggest that D1 mediated signals within the NAc core during a cued-discrimination task do not aid in updating the reward-predictive significance of cues, but rather serve to augment instrumental responding (Calaminus and Hauber, 2007). Thus, it is possible that BLA activity in response to reward-predictive cues drives post-synaptic signals within the NAc core, augmenting coincident phasic dopamine signals to amplify neural and behavioral responses to motivationally significant cues.
CHAPTER 4
BASOLATERAL AMYGDALA REGULATION OF NUCLEUS ACCUMBENS
CELLULAR SIGNALING DURING COCAINE SELF-ADMINISTRATION

ABSTRACT

The neural processing of information regarding successful reward procurement involves a diverse network of brain substrates. The nucleus accumbens (NAc) and the basolateral amygdala (BLA) are vital to associative reward processes, and recent research has examined the functional importance of BLA-NAc interactions. Here, multi-neuron extracellular recordings of NAc neurons coupled to microinfusion of GABA_A and GABA_B agonists into the BLA was employed to determine the functional contribution of the BLA to phasic neural activity within the NAc during cocaine self-administration, and non-contingent presentation of drug-associated stimuli. Results indicate that NAc neurons that encode the lever press response as well as information about cocaine-associated stimuli paired with the response (types RFe and RFi; see (Carelli, 2000; Carelli and Ijames, 2000)) are regulated by the BLA. Specifically, cells that increased their firing rate following the cocaine reinforced response (type RFe) exhibited an attenuated excitatory signal following BLA inactivation. Cells that exhibited a phasic decrease in firing rates surrounding the cocaine reinforced response (type RFi) showed a significant reduction in the baseline firing rate following BLA inactivation. This reduction in baseline activity resulted in a decreased signal-to-noise ratio thus decreasing the magnitude of the phasic response. Additionally, the responsiveness of each cell type to CS probes was also significantly altered as a result of BLA inactivation.
Interestingly, BLA inactivation had no effect on neurons that exhibited an ‘anticipatory’ response profile, increasing activity in the seconds preceding the operant response (type PRe). These findings are consistent with our prior work showing that BLA neurons exhibit similar types of neuronal firing patterns as NAc neurons during cocaine self-administration (Carelli et al., 2003). Together, these results suggest that an excitatory projection from the BLA contributes to conditioned neural excitations within the NAc during cocaine self-administration, while also regulating the tonic firing rate of phasic inhibitions.
INTRODUCTION

Cocaine addiction is marked by explicit control of drug-seeking behaviors by stimuli associated with prior drug use. In human addicts such conditioned stimuli (e.g., drug paraphenila etc) can elicit drug craving and often result in drug-seeking behaviors even after periods of prolonged abstinence from drug use (O'Brien et al., 1998; Childress et al., 1999). Understanding the neural circuitry that mediates the control of reward seeking behavior by conditioned stimuli is essential to developing sufficient treatments and targets for the prevention of drug abuse. Further, it is critical to view the neural circuitry that underlies cocaine-seeking behaviors in relation to the normal circuitry that mediates natural reward processes (Kelley and Berridge, 2002).

Accumulating evidence suggests that the ability of motivationally salient stimuli to augment instrumental responding is reliant on a diverse network of neural substrates, including the nucleus accumbens (NAc) (Wise, 1998; Cardinal and Everitt, 2004; Hyman et al., 2006). The nucleus accumbens (NAc) has long been described as an important neural substrate for reward processing, particularly in mediating the effects of motivationally relevant stimuli on goal-directed responding (Nicola, 2007; Yin et al., 2008a; Humphries and Prescott, 2009). The NAc is a heterogenous structure, comprised primarily of two the anatomically and functionally distinct subregions, the core and shell (Zahm and Brog, 1992; Brog et al., 1993). Both of these structures are involved in processing drug rewards, although there are apparent differences in the processing of conditioned cues (Ito et al., 2004), dopamine signaling during the formation of cocaine-cue associations (Aragona et al., 2009) and differences in reinstatement behaviors (Fuchs et al., 2004). Correspondingly, neural correlates within the NAc have been examined through electrophysiological studies,
revealing that NAc neural activity tracks cocaine-seeking behaviors (Peoples et al., 1997; Carelli and Ijames, 2000; Hollander and Carelli, 2005), reflects cue-drug associations (Carelli, 2000; Ghitza et al., 2004; Hollander and Carelli, 2007), and can be dramatically altered by chronic cocaine exposure (Peoples et al., 1999; Peoples and Cavanaugh, 2003; Peoples et al., 2007). Furthermore, several studies have demonstrated that encoding of cocaine associated cues is augmented as a function of abstinence from drug (Ghitza et al., 2004; Hollander and Carelli, 2007) However, the fundamental contribution of particular afferents to NAc neural activity following drug exposure remains largely unknown.

One critical afferent that has received increasing attention regarding its role in reward processing is the basolateral amygdala (BLA) (Everitt et al., 2000; Murray, 2007). Electrophysiological studies have revealed that BLA neurons are responsive to reward-predictive cues (Tye et al., ; Carelli et al., 2003; Saddoris et al., 2005; Tye and Janak, 2007; Ambroggi et al., 2008), and that cocaine-induced decision making deficits are mediated by miscoding of neural responses within the BLA (Stalnaker et al., 2007). Further, intact BLA function is necessary for the formation of a conditioned place preferences (Everitt et al., 1991; McDonald and White, 1993), Pavlovian-to-Instrumental Transfer (Corbit and Balleine, 2005), cue-induced reinstatement (Fuchs et al., 2006), second-order conditioning (Everitt et al., 1989; Setlow et al., 2002a), reward devaluation (Ostlund and Balleine, 2008; Johnson et al., 2009), and responding on high effort tasks (Simmons and Neill, 2009).

The functional importance of the BLA-NAc circuit has become increasingly evident (Cardinal et al., 2002). Recent evidence has demonstrated that direct interactions between the BLA-NAc core are necessary for cocaine-seeking behavior controlled by cocaine-paired stimuli (Di Ciano and Everitt, 2004). Additionally, recent evidence has shown that stimulus-
controlled instrumental responding is also attenuated as a result of BLA-NAc disconnection (Ambroggi et al., 2008). Neurophysiological evidence has also revealed that stimulation of BLA efferents evoke excitatory responses in NAc neurons (O'Donnell and Grace, 1995; Floresco et al., 2001c; Charara and Grace, 2003; Ambroggi et al., 2008; McGinty and Grace, 2008). Furthermore, BLA activity regulates the phasic terminal release of dopamine within the NAc core evoked by reward-predictive cues (Jones et al., 2009). Interestingly, large populations of NAc neurons exhibit coordinated activity, as measured with local field potential recordings (Berke et al., 2004; Masimore et al., 2005; van der Meer and Redish, 2009), and strong neuronal coherence exists between the amygdala and ventral striatum, allowing for increased signal processing between these neural substrates (Popescu et al., 2009).

Here, we used multi-unit extracellular electrophysiology of NAc activity coupled to microinfusion of GABA_A and GABA_B agonists (muscimol 0.03 nmol and baclofen 0.3 nmol in 0.3µL) (McFarland and Kalivas, 2001) into the BLA, to determine the functional contribution of the BLA to phasic neural activity within the NAc during cocaine self-administration, and during non-contingent presentation of the cocaine-associated stimuli. These data provide a novel characterization of BLA mediated NAc signaling in animals exposed to cocaine, aiding in the understanding of the neuroadaptations associated with chronic drug exposure. Together, these results give a broader perspective on associative mechanisms within the neural circuitry mediating reward processing and insight into underlying factors that result in aberrant reward-seeking behaviors such as drug addiction.
METHODS

Animals

Male Sprague Dawley rats (Harlan Sprague Dawley, Indianapolis, IN) aged 90–150 d and weighing 250–350 gm were used as subjects and individually housed with a 12:12 light:dark cycle. All experiments are conducted between 7:00 am and 7:00 pm. For cocaine self-administration, animals were food restricted to 90-95% pre-surgery body weight, and to no less than 85%. This regimen was in place for the duration of behavioral testing, except during the post-operative recovery period, when food was given ad libitum. All procedures were approved by the University of North Carolina at Chapel Hill Institutional Animal Care and Use Committee.

Experimental Chamber

Behavioral sessions were conducted in a 43 x 43 x 53 cm Plexiglas chambers housed within sound-attenuated cubicles (Med Associates, Albans, VT). One side of the chamber contained two retractable levers (Coulbourn Inst., Allentown, PA) located 17 cm apart. A stimulus light was positioned 6 cm above each lever. A speaker to deliver white noise (80 dB) was mounted 12 cm above the floor on the wall opposite the levers. A houselight (100 mA) was mounted 6 cm above the white noise speaker. For sucrose reinforcement, sucrose pellets (45mg) were delivered to a receptacle located equidistantly between the retractable levers. For cocaine self-administration sessions, a fluid injection assembly (syringe pump) was connected to a swivel system in the experimental chambers that enabled intravenous infusion of cocaine. All environmental events were controlled via a dedicated Pentium computer and commercially available software (Med Associates, Albans, VT).
Surgery

Animals were anesthetized with ketamine hydrochloride (100 mg/kg) and xylazine hydrochloride (20 mg/kg), and microelectrode arrays were implanted in the NAc using established procedures (Carelli et al., 2000). Electrodes were custom-designed and purchased from a commercial source (NB Laboratories, Dennison, TX, USA). Each array consisted of eight microwires (50 µm diameter) arranged in a 2x4 bundle that measured ~ 1.5 mm anteroposterior and ~ 0.75 mm mediolateral. Arrays were targeted for permanent, bilateral placement in the core and shell subregions of the NAc (AP, +1.3 to 1.7 mm relative to bregma; ML, ± 0.8 or 1.3 mm relative to bregma; DV, −6.2 mm from brain surface; (Paxinos and Watson, 2005)). Ground wires for each array were coiled around skull screws and placed into the ipsilateral side of the brain. Rats were also implanted with bilateral guide cannula aimed at the BLA (anteroposterior: -2.7 to -3.0; mediolateral: +/-4.9 from bregma; dorsoventral: -6.2 from skull, (Paxinos and Watson, 2005)). BLA guide cannula consisted of a 22 gauge thin-walled stainless-steel tubing (Plastics One Inc., Roanoke, VA) and were lowered to 2 mm above the target site. Rats were also implanted with a catheter into their jugular vein for cocaine self-administration, using established procedures (Carelli and Ijames, 2000; Hollander and Carelli, 2007). The catheter was routed subcutaneously to the back and attached to a coupling assembly. Following surgery rats were given a minimum of 5 days of recovery.

Behavioral training
Following surgery, rats underwent 5-7 days of operant training for sucrose reinforcement. These sessions were used to habituate animals to the operant chambers and to train them to press a lever for reinforcement, prior to initiation of cocaine self-administration sessions. Animals were reinforced on a continuous reinforcement schedule on both levers, wherein a single response on either the left or right lever resulted in sucrose pellet delivery. Rats were given 30 min sessions or a maximum of 100 operant responses (50 per lever).

Next, rats were trained to self-administer cocaine during daily 2 h sessions in the experimental chamber. The beginning of the self-administration session was signaled by the onset of a cue-light above the active lever and lever extension. The alternate inactive lever was also extended, but the cue-light above it was not illuminated. Active lever responses were reinforced on a fixed-ratio 1 (FR1) schedule, wherein a single response resulted in intravenous cocaine delivery (0.16 mg/infusion dissolved in sterile heparinized saline vehicle (Carelli and Deadwyler, 1996)) over a 6 s period via a computer controlled syringe pump (Model PHM-100, Med Associates, Inc., St. Albans, VT). Initiation of drug delivery was signaled by termination of the cue-light and simultaneous onset of a compound conditioned stimulus (CS) consisting of a tone (67 db, 1 kHz) house-light stimulus complex (20 s). Responses made on the lever during the CS were not reinforced, but were recorded. Cocaine self-administration training was completed when stable responding was established following a minimum of 14 training sessions. Stable self-administration was defined as at least two days of responding where the standard error of the inter-trial interval (ITI) was less than one half the mean. Following each session, catheters were flushed with heparinized saline.

*Test sessions*
The experimental test session was divided into three phases. NAc activity was recorded during each phase. The pre-infusion phase (PRE) was identical to a regular training session described above. The PRE phase length was set to a limit of 2h, with a minimum of 1h duration. If the animal reached the desired criterion of 20 lever responses before the end of 2h this phase was terminated and the next phase initiated. At the completion of the PRE phase, rats were immediately moved to the next phase.

The second and third phases consisted of the BLA manipulations, wherein rats were infused with either VEH or BM unilaterally into the BLA (VEH and BM phases respectively). Following the infusion, rats were given 5 minutes for recovery before the cue-light above the active lever was illuminated and the levers were extended. The infusion phases then proceeded with the same behavioral procedure used in the PRE phase, except animals were allowed to self-administer during a 2 h session. At the conclusion of the first infusion phase, the cue-light was terminated and the levers were retracted, and rats received the alternate infusion into the BLA, and an additional 2 h self-administration period was initiated.

To examine the responsiveness of NAc neurons to the CS paired with each cocaine infusion during self-administration sessions, the ‘CS probe’ procedure was used (Carelli, 2000). Specifically, the CS tone-houselight stimulus complex (that was always paired with cocaine infusion during self-administration during training) was randomly presented by the experimenter during the inter-infusion interval of the self-administration session. Specifically, CS probes (20s) were delivered variably, with a minimum of 60 s between probes, and minimum of 30 s after a reinforced response. During the CS probes, responses on the active lever were not reinforced.
BLA microinfusions

Intracranial microinfusions were made through a 28 gm injector (Plastics One Inc., Roanoke, VA), which was lowered to the site of injection, 2 mm below the guide cannula. Infusions consisted of 0.3 µl of either vehicle (VEH; 0.9% sterile saline) or drug (BM; baclofen hydrobromide/muscimol dissolved in 0.9% sterile saline; 0.3nmol/.03nmol respectively; Sigma Aldrich)(McFarland and Kalivas, 2001; Rizos et al., 2005) delivered over 60 seconds to the unilateral BLA using a syringe pump (Harvard Apparatus, Holliston, MA), followed by a 60 sec post-infusion diffusion time. Following the infusion, the rat was returned to the testing chamber for 5 minutes before testing continued.

Neural analysis

NAc neuronal firing patterns were characterized using raster displays and peri-event histograms (PEHs) constructed with commercially available software (NeuroExplorer, Plexon). For each cell, two PEHs were constructed. The first PEH was synchronized to the cocaine-reinforced lever press response and contained three analysis epochs including: (1) a 'baseline' period (−10 to 5 s prior to reinforced lever response); (2) a pre response event period (−2 -0 s preceding the operant response) and (3) a post response event period (0 - 2 s following the operant response). The second PEH was synchronized to the CS probe trials and contained two analysis epochs including: (1) a baseline period (-5 – 0 s before CS probe); and (2) the CS probe event period (0-2s following probe onset). The activity of each cell was scored based upon its firing rate within the event epochs. Specifically, 99.9% confidence intervals (CI) were projected from the baseline epochs of each neuron in the
PEHs (Neuroexplorer). Neural responses were characterized as phasic by the presence of at least one bin (beginning within each event epoch) that showed an increase or decrease in firing rate over the projected baseline CI. A subset of cells exhibited larger variability in their baseline firing rates and the projected 99.9% CI included 0. For these neurons to be classified as phasic, $e_0 > 2b_0$ had to be true (where $e_0$=consecutive zero bins occurring within the event epoch, and $b_0$= maximal number of consecutive zero bins in the baseline epoch).

Next, NAc neurons were classified into specific response profiles, as in prior studies (Hollander and Carelli, 2007), as follows. Cells that increased their firing rate before the operant response, were classified as Pre-response (PRe) neurons, even if the phasic increase persisted after response completion. Cells that increased their firing rate immediately following the response were classified as type ‘Reinforcement-Excitation’, or RFe neurons. Note, PRe and RFe are inherently mutually exclusive. Cells that decreased their firing rate surrounding the operant response were classified as type ‘Reinforcement-Inhibition’, or RFi neurons. Another subset of cells, termed type ‘PR+RF’ cells exhibited dual increases in firing rate both before and after the response with a marked inhibition between the two events. Finally, cells that exhibited significant increases or decreases in firing rate relative to CS probe onset were termed type CSe or CSI respectively.

**Neural exclusion criteria**

Two exclusion criteria were used to remove units that would not meet standards for NAc neurons. Cells were excluded if the baseline firing rate for the PRE period was less than 0.1 Hz or if the cell had fewer than 500 spikes within this period. Cells were also
removed from analysis if the baseline firing rate during the PRE period exceeded 10 Hz, as these were likely not medium-spiny neurons (Berke et al., 2004).

**Histology.**

Rats were deeply anesthetized with a ketamine and xylazine mixture (100 mg/kg and 20 mg/kg, respectively) and perfused transcardially using physiological saline and 10% formalin and 3% potassium ferricyanide, and brains were removed. In order to mark the placement of electrode tips, a 13.5 µA current was passed through each microwire electrode for 5 s. To mark microinfusion sites, brief 5 µA current was passed through injector needles placed into the guide cannula. After post-fixing and freezing, 40-µm coronal brain sections were mounted and stained with thionin. Electrode tip locations and injection sites were identified based upon anatomical organization (Paxinos and Watson, 2005).

**Statistics**

For behavioral analysis, a two-way repeated measures ANOVA of lever (active v inactive) x session (1-10) was used to compare reinforced responding over training. Cumulative activity graphs were used to assess lever press responding. To compare VEH and BM treatments on reinforced responses, a paired t-test was employed. One-way repeated measures ANOVAs were employed to examine average ITI or latency to first press across test phase. Bonferroni post hoc tests were employed as appropriate.

For analysis of neural data, proportions and frequencies were compared using Fisher’s exact test. To compare the average firing rate of subpopulations of NAc neurons, paired t-tests compared the baseline versus signal epoch. To determine the effects of BLA
manipulation on cell firing, PEHs were constructed for each individual neuron across the VEH and BM infusion periods to compare within-session changes in firing rate. Cells were grouped based on their hemispheric relation to the infusion side. To examine changes within each cell group, a two-way repeated measures ANOVA of epoch (baseline v signal) x treatment (VEH v BM) was used. Statistics were analyzed using either Prism 4 (GraphPad) SPSS 17.0 (SPSS).
RESULTS

Histology

Histological verification of electrode placements revealed that a total of 49 wires were located within the NAc. Likewise, eight micro-infusion sites were histologically confirmed to be within the BLA. Non-NAc wire placements were excluded from analysis. Figure 4.1 is a schematic representation of the NAc wire placements and the BLA placements.

Figure 4.1. Histological representation of electrode and cannula placement. Left diagrams show the NAc placements of electrodes at recording. Black circles are accepted NAc placements (n=49 and grey circles are missed locations (n=15). Right diagrams illustrate the tip of the microinjector placed into the BLA cannula (Paxinos, 2005)

Acquisition of cocaine self-administration and the effects of BLA manipulation

Figure 4.2a is a schematic diagram of the behavioral paradigm. Rats (n=4) readily learned to discriminate between the active and inactive levers across the training sessions. For comparison across similar time-points, the final 10 training sessions were compared. A
two-way ANOVA (lever x session) revealed a significant main effect of lever (F(1,3) = 22.4, p=0.018) across the final 10 training sessions (Figure 4.2b). The final day of training was marked by a total of 36±10 reinforced responses and 0.25±0.25 inactive responses, and a mean ITI of 227.1 s ±43.9 for reinforced responses.

To examine the effects of unilateral BLA manipulation on cocaine self-administration, Figure 4.2c illustrates a representative cumulative activity graph for reinforced responses across the three phases of the test session. BLA treatment with BM had no significant influence on the pattern of cocaine self-administration. The total reinforced responses across the VEH (30.4±7.5) and BM (32.75±7.97) sessions were not significantly different (t(7)=0.55, p=0.60; Figure 4.2d). To determine whether BLA manipulation affected responding, a one-way repeated measures ANOVA compared the PRE, VEH and BM ITIs. There was a significant effect of phase on the average ITI (F(2,12) = 3.89, p=0.0499; Figure 4.2e), largely driven by the shorter ITI of the PRE phase. Importantly, post hoc analysis revealed there was no significant difference between the VEH and BM phases. Additionally, one-way repeated measures ANOVA revealed that there was no significant effect of phase on the latency to first press (F(2,12) = 0.71, p=0.51; Figure 4.2f).
Figure 4.2. Behavioral task and self-administration behaviors following BLA treatment. a) Schematic of the operant task. During sessions, rats could respond on the active lever (L*) (indicated by white triangle) for intravenous cocaine (infusion period indicated by black horizontal line). The inactive lever (L^i) was always present; responses on it were never reinforced (Black triangles indicate nonreinforced presses). Intermittently during the ITI, non-contingent CS probe trials were administered. Responses during CS probes had no programmed consequences. b) Average number of lever presses across the final ten training sessions. c) Cumulative activity graph from a representative animal across the PRE (black), VEH (pink), and BM (tan) phases. d) Average number of reinforced responses of all animals across VEH and BM treatments. e) Average ITI of all animals across the PRE, VEH and BM phases. f) Average latency to first reinforced press for all animals across the PRE, VEH and BM phases. Data and error bars reflect mean ± SEM. ns denotes p>0.05; * denotes p<0.05.

Characterization of NAc neural activity

A total of 71 NAc neurons were recorded across eight recording sessions. Neurons were assigned to a specific type of neuronal firing pattern based on the changes in firing rate surrounding the reinforced response or the CSp presentation (see Methods). Table 4.1 shows the number of neurons that exhibited one of these phasic firing patterns. There were four types of phasic response patterns surrounding the reinforced response, and two types of phasic response patterns in response to the CSp. Of the 71 neurons recorded, 40 (56.3%) exhibited at least one phasic response profile during this task. This number is not reflective of
the sum of phasic responses in the table a given cell can express more than one phasic profile (e.g. RFe and CSe).

<table>
<thead>
<tr>
<th>Phasic Response</th>
<th>Total (n=71)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
</tr>
<tr>
<td>Pre</td>
<td>9</td>
</tr>
<tr>
<td>RFe</td>
<td>10</td>
</tr>
<tr>
<td>RFi</td>
<td>18</td>
</tr>
<tr>
<td>PR+RF</td>
<td>1</td>
</tr>
<tr>
<td>CSe</td>
<td>12</td>
</tr>
<tr>
<td>CSi</td>
<td>4</td>
</tr>
</tbody>
</table>

Examples of three of the four types of patterned discharges observed relative to the reinforced response are presented in Figure 4.3. The PEH and raster display shown in Fig. 4.3a illustrates the activity of a representative neuron classified as a type PRe cell (it showed a significant increase in firing rate during the 2s preceding the reinforced response). For all type PRe cells, there was a significant increase in signal versus baseline firing rates ($t_{(8)}=3.32$, $p=0.011$, Fig. 4.3b, right). Other neurons were characterized by significant increases in firing rate during the 2s following the reinforced response (type RFe). An example of the activity of a representative RFe cell is shown in Fig. 4.3c. There was a significant difference in the average baseline and signal firing rate for all RFe neurons ($t_{(9)}=3.61$, $p=0.006$, Fig. 4.3d). Neurons that were characterized by decreases in firing rate surrounding the reinforced response were labeled as RFi; an example is shown in Fig. 4.3e. As with other cell types, the average signal firing rate was significantly less than baseline activity for all RFi neurons ($t_{(17)}=4.99$, $p=0.0001$, Fig. 4.3f).
Figure 4.3. A subset of NAc neurons exhibit phasic changes in cell firing relative to the cocaine reinforced response during self-administration. a) PEH and raster display show the activity of a representative PRe neuron; activity is aligned to the reinforced response (time 0s) here and for other cell types below. PRe epoch (signal period) is denoted by the gray bar. b) Average firing rate across baseline and signal periods for all PRe neurons. c) PEH and raster display show the activity of a representative RFe neuron; activity. RFe epoch (signal period) is denoted by the gray bar. d) Average firing rate across baseline and signal periods for all RFe neurons. e) PEH and raster display show the activity of a representative RFi neuron. RFi epoch (signal period) is denoted by the gray bar. f) Average firing rate across baseline and signal periods for all RFi neurons. Data and error bars reflect mean ± SEM. ** denotes p<0.01; * denotes p<0.05.

The fourth response pattern was much less common, but was characterized by two independent response phases; a significant increase in firing rate preceding the reinforced response, and a significant increase in firing rate following the reinforced response, separated by a return to baseline firing (type PR+RF). Figure 4.4 shows the activity of this neuron.
Figure 4.4. A single NAc neuron exhibited the PR+RF response pattern relative to the reinforced response. PEH and raster display show the activity of this neuron aligned to the reinforced response (time 0s). PR+RF epoch (signal period) is denoted by the gray bar.

*Activation of NAc neurons by CS probes*

The responsiveness of NAc neurons during CS probe presentation was also determined. Neurons that exhibited significant increases in firing rate following CS probe onset were labeled type CSe. For all CSe cells, there was a significant increase in average firing rate during the signal versus baseline periods ($t_{(11)}=2.96$, $p=0.013$, Fig 4.5b). Of the 12 CSe neurons, a majority ($n=7$) were also characterized as RFe neurons, and a minor proportion were characterized as PRe cells ($n=3$), or were only phasic to the CS probe ($n=2$). Neurons that exhibited significant decreases in firing rate following CS probe onset were labeled as type CSi. The average firing rate during the signal period was significantly less than activity during baseline for all CSi neurons ($t_{(3)}=3.28$, $p=0.046$, Fig 4.5d). Of the 4 CSi neurons, half ($n=2$) were also characterized as RFi, one was characterized as PRe, and the remaining neuron was only responsive to the CS probe. Interestingly, a greater proportion of neurons exhibited CSe ($n=12$) as compared to CSi ($n=4$) response patterns (Fisher’s exact test; $p=0.036$).
Figure 4.5. A subset of NAc neurons exhibited phasic changes (increases or decreases) in cell firing relative to CS probe presentation. a) PEH and raster display show the activity of a representative CSe neuron; activity is aligned to the CS probe onset (left raster, time 0s) and also to the reinforced response (right raster; time 0s). CSe epoch (signal period) is denoted by the gray bar. b) Average firing rate across baseline and signal periods for all CSe neurons. c) PEH and raster display show the activity of a representative CSi neuron; activity is aligned to the CS probe onset (left raster, time 0s) and also to the reinforced response (right raster; time 0s). CSi epoch (signal period) is denoted by the gray bar. b) Average firing rate across baseline and signal periods for all CSi neurons. Data and error bars reflect mean ± SEM. * denotes p<0.05.

*BLA effects on NAc cell firing during cocaine self-administration*

To determine the effect of BLA treatment on NAc cell firing relative to the reinforced response, each specific subset of phasic responses was examined individually. First, the effect of BLA inactivation on PRe response profiles was examined. A total of 9 PRe neurons were recorded across the sessions. Two neurons were located ipsilaterally to the BLA treatment, while the remaining seven were located contralaterally to BLA treatment. Thus, only contralateral neurons were analyzed. A two-way repeated measures ANOVA treatment (VEH v BM) x epoch (baseline v signal) revealed no significant main effect of treatment...
(F(1,6) = 2.08, p>0.05), no main effect of epoch (F(1,6) = 4.167, p>0.05), and no significant interaction (F(1,6) = 5.72, p>0.05). Thus, the activity of type PRe neurons was not altered by BLA inactivation (data not shown).

A total of 10 RFe neurons were recorded across all sessions. Six of these neurons were located ipsilaterally to the BLA treatment, while the remaining four were located contralaterally to the BLA treatment. Figure 4.6 shows the average firing rate of all RFe neurons following VEH and BM treatment located ipsilateral (Figure 4.6a) or contralateral (Figure 4.6b) to the infusion. RFe neurons recorded ipsilaterally to BLA treatment exhibited a significant attenuation in the magnitude of the RFe signal. A two-way repeated measures ANOVA treatment (VEH v BM) x epoch (baseline v signal) revealed a significant main effect of epoch (F(1,5) = 7.93, p=0.04), but not treatment (F(1,5) = 2.38, p>0.05), but a significant interaction (F(1,5) = 8.78, p=0.03) (Figure 4.6c). Importantly, RFe neurons that were contralateral to the BLA manipulation were not significantly attenuated by BM treatment. A two-way repeated measures ANOVA treatment (VEH v BM) x epoch (baseline v signal) revealed a significant main effect of epoch (F(1,3) = 13.411, p=0.04), but not treatment (F(1,3) = 0.06, p>0.05), and no significant interaction (F(1,3) = 3.13, p>0.05) (Figure 4.6d).
Figure 4.6. Effect of BLA treatment on RFe activity. Average traces of all RFe neurons ipsilateral (a) and contralateral (c) to BLA treatment during both VEH (pink) and BM (tan) treatment. Traces are aligned to the reinforced response (time 0s). Average firing rate during the baseline and signal epochs for all RFe neurons ipsilateral (d) and contralateral to the BLA infusion during VEH and BM treatments. Data and error bars reflect mean ± SEM. * denotes p<0.05.

A total of 18 RFi neurons were recorded across all sessions. Figure 4.7 shows the average firing rate of all RFi neurons following VEH and BM treatment located ipsilateral (Figure 4.7a) or contralateral (Figure 4.7b) to the infusion. RFi neurons that were ipsilateral to the BLA treatment exhibited a significant attenuation in baseline firing rate. A two-way repeated measures ANOVA treatment (VEH v BM) x epoch (baseline v signal) revealed a significant main effect of epoch ($F_{(1,11)} = 14.28$, $p=0.003$), but not treatment ($F_{(1,11)} = 1.095$, $p>0.05$), but a significant interaction ($F_{(1,11)} = 14.15$, $p=0.003$) (Figure 4.7c). Note that the net effect of this attenuation in baseline firing was a reduction in the magnitude RFi activity. Importantly, RFi neurons that were contralateral to the BLA manipulation were not significantly attenuated as a function of BM treatment. A two-way repeated measures ANOVA treatment (VEH v BM) x epoch (baseline v signal) revealed a significant main
effect of epoch \( (F_{(1,5)} = 6.86, \ p=0.047) \), but not treatment \( (F_{(1,5)} = 1.49, \ p>0.05) \), and no significant interaction \( (F_{(1,5)} = 0.062, \ p>0.05) \) (Figure 4.7d).

![Image](image_url)

**Figure 4.7.** Effects of BLA treatment on RFi activity. Average traces of all RFi neurons ipsilateral (b) and contralateral (b) to BLA treatment during both VEH (pink) and BM (tan) treatment. Traces are aligned to the reinforced response (time 0s). Average firing rate during the baseline and signal epochs for all RFi neurons ipsilateral (c) and contralateral (d) to the BLA infusion during VEH and BM treatments. Data and error bars reflect mean ± SEM. * denotes \( p<0.05 \).

**BLA effects on CS-evoked neural activity**

Neurons that exhibited a change in firing rate relative to CS probes were classified as either type CSe or type CSi neurons. A total of twelve CSe neurons were recorded across all sessions. Figure 4.8 shows the average firing rate of all CSe neurons following VEH and BM treatment located ipsilateral \( (n=7; \text{Figure 4.8a}) \) or contralateral \( (n=5; \text{Figure 4.8b}) \) to the BLA infusion. CSe neurons that were ipsilateral to the BLA treatment exhibited a significant decrease in the CSe signal. A two-way repeated measures ANOVA treatment \( (\text{VEH v BM}) \) x epoch \( (\text{baseline v signal}) \) revealed a significant main effect of epoch \( (F_{(1,6)} = 8.31, \ p=0.03) \),
but not treatment \( (F_{(1,6)} = 3.61, p>0.05) \), but a significant interaction \( (F_{(1,6)} = 6.67, p=0.042) \) (Figure 4.8c). Importantly, the activity of CSe neurons recorded contralateral to the BLA manipulation was not significantly attenuated as a function of BM treatment. A two-way repeated measures ANOVA treatment (VEH v BM) x epoch (baseline v signal) revealed no significant main effect of epoch \( (F_{(1,4)} = 3.59, p>0.05) \), but not treatment \( (F_{(1,4)} = 0.56, p>0.05) \), and no significant interaction \( (F_{(1,4)} = 0.72, p>0.05) \) (Figure 4.8d).

![Figure 4.8](image)

**Figure 4.8.** Effects of BLA treatment on CSe activity. Average traces of all CSe neurons ipsilateral (a) and contralateral (b) to BLA treatment during both VEH (pink) and BM (tan) treatment. Traces are aligned to the CS probe onset (time 0s). Average firing rate during the baseline and signal epochs for all CSe neurons ipsilateral (c) and contralateral (d) to the BLA infusion during VEH and BM treatments. Data and error bars reflect mean ± SEM. * denotes \( p<0.05 \).

As only four neurons were characterized as CSi across all sessions, statistical analysis differentiating between neurons that are ipsilateral versus contralateral to the BLA treatment were not run. However, Figure 4.9 shows that a similar trend is seen in the CSi neurons ipsilateral to the BLA treatment, similar to RFi neurons. That is, there appears to be a
decrease in the baseline firing rate in CSi neurons resulting in a decrease in the magnitude of the inhibitory signal during CS probes for this population of cells, which is appropriate as these neurons also displayed RFi response patterns.

Figure 4.9. Effects of BLA treatment on CSi activity. Graph illustrates the average firing rate during the baseline and signal epochs for the two CSi neurons ipsilateral to the BLA infusion during VEH and BM treatments.
DISCUSSION

The present findings demonstrate that the BLA differentially regulates the activity of distinct populations of NAc neurons during cocaine self-administration. Specifically, NAc neurons that encode the lever press response as well as information about cocaine-associated stimuli paired with the response (types RFe and RFi; see (Carelli, 2000; Carelli and Ijames, 2000)) were altered by BM inactivation of the BLA. This was in the form of a reduction in the excitatory profile of type RFe cells, and a reduction in the baseline firing rate of type RFi cells. For RFi cells, this reduction in baseline activity lead to a decreased signal-to-noise ratio thus decreasing the magnitude of the phasic response. Additionally, the responsiveness of these cell types to CS probes was also significantly altered as a result of BM inactivation. Interestingly, BLA inactivation had no effect on neurons that exhibited an ‘anticipatory’ response profile, increasing activity in the seconds preceding the operant response (type PR). These findings are consistent with our prior work showing that BLA neurons exhibit similar types of neuronal firing patterns as NAc neurons during cocaine self-administration (Carelli et al., 2003), and demonstrate that the excitatory projection from the BLA contributes to conditioned neural excitations and inhibition of select populations of NAc neurons during cocaine self-administration.

The primary finding reported here is that distinct subsets of NAc neurons are differentially regulated by BLA activity during cocaine self-administration. Recent evidence has demonstrated that BLA activity facilitates incentive cue-responses of NAc neurons, concomitant with a decrease in stimulus-controlled behavior (Ambroggi et al., 2008). Those authors examined responding for a natural reward however, and also utilized a discriminative-stimulus task. Here, we demonstrate that pharmacological inactivation of the
BLA also attenuates conditioned neural excitations as evidenced by alterations in the ability of CS probes to activate NAc activity following BLA inactivation. Interestingly, neural activity surrounding the reinforced response, specifically post-response excitations, was also attenuated. Such post-response excitations are typically associated with encoding of the CS that was always paired with cocaine infusion during self-administration (Carelli and Deadwyler, 1997; Carelli, 2000). Those results were replicated here, as a majority of RFe neurons were also responsive to non-contingent CS probes. Moreover, CSi responses evoked by non-contingent probes were also significantly attenuated by BM treatment of the BLA. Critically, both of these response patterns were altered by ipsilateral, but not contralateral, inactivation demonstrating a predominant ipsilateral BLA regulation of NAc activity.

Likewise, NAc neurons that exhibited phasic decreases in firing rate surrounding the reinforced response (type RFi cells) were also affected by BLA inactivation. However, rather than the signal being altered, the baseline firing rate of these neurons were reduced, effectively reducing the signal-to-noise ratio for RFi cells. This finding suggests that tonic BLA activity selectively contributes to the baseline firing rate of this subset of NAc neurons. Again, this result was specific to neurons ipsilateral to the BLA infusion. Interestingly, this result is not seen in phasic inhibitions when an animal was responding for a sucrose reward (Chapter 3). It is possible that this is different under cocaine reinforcement because the pharmacological effect of cocaine significantly reduces the overall firing rates of NAc neurons (White et al., 1993; Peoples et al., 1998). Thus, once the baseline is already attenuated through the pharmacological action of the drug, the normally minor contribution of BLA activity to the baseline firing rate becomes more significant.
These results are consistent with anatomy studies which show that the BLA sends primarily glutamatergic efferents to the NAc (Kelley et al., 1982; McDonald, 1991; Brog et al., 1993). Previous electrophysiological studies have shown that stimulation of BLA efferents evoke excitatory responses in NAc neurons (O'Donnell and Grace, 1995; Floresco et al., 2001c; Charara and Grace, 2003; Ambroggi et al., 2008; McGinty and Grace, 2008). Furthermore, while BLA neurons do not exhibit tonically high firing rates, it is plausible that the tonic activity of BLA neurons could contribute to the baseline firing rate of NAc neurons. Electrophysiological studies have revealed that BLA neurons are responsive to reward-predictive cues (Tye et al., Carelli et al., 2003; Saddoris et al., 2005; Tye and Janak, 2007; Ambroggi et al., 2008), and that cocaine-induced decision making deficits are mediated by persistent neural responses within the BLA to cues that are no longer reward-predictive (Stalnaker et al., 2007). The results presented here are consistent with these findings, as BLA inactivation with GABA agonists likely eliminates cue-evoked activity altogether, which would result in an attenuation of downstream signals within the NAc.

It is interesting to consider the effects of BLA inactivation on NAc neural activity in the scope of large population activity. Recent electrophysiological recordings of the local field potentials (LFP) within the ventral striatum have shown that LFP oscillations show coherence with different afferent inputs. Typically, 50 Hz gamma oscillations predominate in the NAc, and this is primarily coherent with amygdala activity (Popescu et al., 2009), whereas periods of high frequency 80-100 Hz gamma oscillations occur and are coherent with the frontal cortex (Berke, 2009; van der Meer and Redish, 2009). This high frequency gamma oscillation is also responsible for entraining activity of fast spiking interneurons (presumably GABAergic) (Berke et al., 2004; Berke, 2008, 2009). Administration of
amphetamine or dopamine agonists cause a prolonged increase in the cortex coherent gamma frequency (Berke, 2009). Presumably, this increased 80 Hz coherence will increase interneuron release of GABA onto MSNs decreasing their overall activity. Moreover, transient shifts to 50 Hz gamma oscillations (amygdala-mediated) signal movement initiation in regions of the striatum (Masimore et al., 2005), and this gamma frequency coherence in the ventral striatum is abolished with amygdala inactivation (Popescu et al., 2009). While these results measure different neurophysiological signals, our results are consistent with LFP studies, in which the importance of amygdala-striatal coherence is evident.

Importantly, unilateral inactivation of the BLA did not significantly alter cocaine self-administration on this relatively simply instrumental task. Animals were only required to respond on an FR1 schedule. BLA alteration of behavioral responding is typically only observed in tasks where explicit cues augment or elicit instrumental responses. For example, intact BLA function is necessary for the formation of a conditioned place preferences (Everitt et al., 1991; McDonald and White, 1993), Pavlovian-to- Instrumental Transfer (Corbit and Balleine, 2005), cue-induced reinstatement (Fuchs et al., 2006), second-order conditioning (Everitt et al., 1989; Setlow et al., 2002a), reward devaluation (Ostlund and Balleine, 2008; Johnson et al., 2009), and responding on high effort tasks (Simmons and Neill, 2009). Furthermore, cocaine self-administration behavior on an FR1 schedule is most highly correlated with the level of cocaine (or dopamine levels) elevated within the NAc (Peoples and West, 1996; Nicola and Deadwyler, 2000). Our behavioral design provides us with uninterrupted behavior with which to align our neural data, however, the functional consequence of these attenuated signals is difficult to assay in the current paradigm. Future studies that examine the contribution of BLA activity to ongoing NAc signaling in
reinstatement and PIT tasks will most likely provide the functional consequences correlated with these attenuated neural responses.
CHAPTER 5
GENERAL DISCUSSION

Summary of experiments

The studies described in the previous chapters were designed to elucidate the role of the BLA-NAc circuit in cued-instrumental behaviors involving natural versus cocaine rewards. This was completed by assessing subsecond NAc dopamine release and NAc cellular activity during a variety of behavioral tasks, coupled to circuit-level pharmacological manipulation of neural activity within the BLA. Taken together, the results demonstrate that afferent connections to the NAc provide a dynamic, functionally relevant contribution to associative processing of reward-predictive environmental stimuli. This chapter will briefly review these findings, provide a general discussion of their relevance, and suggest future directions for research endeavors. Taken together, these studies provide a significant advance in our understanding of the functional regulation, and behavioral relevance of the BLA to NAc signaling.

*Phasic dopamine signaling mediated by the BLA*

The results in chapter two provide one of the first characterizations of the ability of NAc afferents to terminally modulate phasic dopamine release, independent of changes in VTA activity. This study employed a discriminative stimulus instrumental task in which one cue predicted access to a lever that when pressed, resulted in sucrose reinforcement. An
additional non-associated cue predicted access to an unrewarded lever. We observed that during this task, the reward-predictive cue elicited robust increases in NAc dopamine concentration in the NAc core. Moreover, BLA inactivation selectively attenuated this cue-evoked dopamine release, without a significant change in dopamine concentration surrounding other behavioral events, or dopamine release evoked by VTA stimulation.

**BLA activity regulates cue-evoked signaling in NAc core neurons**

The results in chapter three provide one of the first characterizations of NAc neural activity regulated by the BLA. Multi-neuron electrophysiological recordings were employed as animals performed a discriminative stimulus instrumental task in which a distinct cue predicted access to a lever associated with sucrose reward. These data provide a critical dissociation of cue-evoked versus operant response patterns through the use of unilateral inactivation of the BLA and temporally distancing the cue from the instrumental response. Furthermore, the data demonstrate that NAc encoding of reward-predictive cues across NAc subregions can be parsed through isolated manipulation of distinct afferent neural substrates. Specifically, encoding of reward-predictive cues in the NAc core, but not the shell, is significantly attenuated by BLA inactivation. These results provide a novel characterization of the ongoing regulation of NAc signaling during cued-instrumental responding for a natural reward.

**BLA activity regulates NAc activity during cocaine self-administration**

The results in chapter four provide the first characterization of afferent regulation of NAc neural activity in cocaine-experienced animals. Animals were trained to self-administer
cocaine on an FR1 schedule of reinforcement, and NAc neural activity was recorded following unilateral manipulation of BLA activity. Neurons exhibiting an increase in firing at the time of the response, which are typically associated with encoding aspects of the CS paired with drug infusion, were significantly attenuated by BLA inactivation. Furthermore, explicit CS excitations evoked by non-contingent probes were significantly attenuated following BLA inactivation. Additionally, inhibitory phasic responses surrounding the operant response exhibited a decreased signal-to-noise ratio, as the baseline firing rate of these neurons was significantly reduced. These results demonstrate that the BLA regulates NAc neural activity during cocaine self-administration, albeit differently than during responding for natural rewards, in that operant inhibitions exhibited robust decreases in baseline firing as a result of BM inactivation.

General discussion and relevance of findings

Although the unique implications of each study are discussed individually within each data chapter, these findings also have further implications for the role of both the NAc and the BLA in associative processing of reward-related stimuli, and how this function may relate to compulsive psychiatric disorders such as drug addiction. Therefore, a brief discussion of these topics is included.

Terminal modulation of phasic dopamine release within the NAc

The first experiment described in this dissertation provides a novel characterization of the regulation of phasic dopamine release within the NAc. The ability to monitor phasic dopamine release on a temporally precise time-scale remains a new capability, and in vivo
monitoring during behavioral tasks has gained significant prominence in recent years (Garris et al., 1997; Phillips et al., 2003c; Roitman et al., 2004; Day et al., 2007). As such, a great deal of information concerning the dynamics of real-time dopamine release, particularly in relation to functional behavioral consequences, remains unknown. For example, it has only recently been demonstrated that phasic dopamine release within the NAc is actually the result of burst firing of dopamine neurons within the midbrain (Sombers et al., 2009). This study infused NMDA-receptor antagonists into the VTA and measured rapid dopamine release within the NAc. While other techniques have suggested that dopamine release within the NAc can be mediated terminally by other afferent inputs (Floresco et al., 1998; Howland et al., 2002; Phillips et al., 2003a), this had not been adequately demonstrated on a functionally relevant timescale. Here, we provide a significant advance in our understanding of the dynamics of NAc dopamine signaling, by showing that afferent connections from the BLA terminally modulate cue-evoked dopamine release within the NAc.

It is generally accepted that dopamine release does not drive post-synaptic signaling of NAc neurons, but rather acts as a neuromodulator, either attenuating or augmenting the ability of afferent input to elicit action potentials in MSNs (Nicola et al., 2000). This modulation is dependent on the present firing rate of the MSN, the extracellular concentration of dopamine, the type of dopamine receptor expressed within the cell, and the temporal relationship of the afferent input (Surmeier and Kitai, 1993; Nicola et al., 2000; Surmeier et al., 2007; Shen et al., 2008). Furthermore, recent technological advances have allowed for simultaneous recording of both subsecond dopamine release and post-synaptic cell firing at the same carbon fiber electrode (Cheer et al., 2007; Owesson-White et al., 2009). These studies show that phasic response patterns in MSNs are often coincident with
sites where rapid dopamine release is also present (Owesson-White et al., 2009). This suggests that dopamine does play a key role in mediating changes in neuronal excitability. As such, the results presented here suggest a mechanism by which afferent inputs (e.g. the BLA) can facilitate VTA-mediated dopamine transients. The presumed consequence of this facilitation would be an augmentation (or general gain-amplification) of afferent-mediated post-synaptic signals within the MSN. The results of chapters 3 and 4 provide evidence that this is likely the case.

*BLA mediation of NAc neural activity: Subregion specificity*

The results in chapter 3 demonstrate that in a cued-instrumental task, wherein a discrete cue predicts access to reinforcement, the BLA differentially regulates conditioned neural responses in the NAc depending on the subregion. Specifically, the BLA regulates cue-evoked excitations in the core, but not the shell subregion. These results merit further discussion. While the attenuation of cue-evoked signaling within the NAc core was predicted, the corresponding lack of effect on shell neurons was surprising for a number of reasons. First, the glutamatergic projection from the BLA to the NAc is not isolated to the core; a topographical, but widespread projection is distributed to both the core and shell (Kelley et al., 1982; McDonald, 1991; Brog et al., 1993; Johnson et al., 1994). There is evidence that a functional rostral-caudal gradient exists (Kantak et al., 2002; McLaughlin and Floresco, 2007), but the topographical organization of the BLA projection to the NAc still exhibits a tremendous amount of overlap, particularly at our target site of manipulation (Shinonaga et al., 1994). Second, neurons in the shell do exhibit robust excitations in response to presentation of reward-predictive cues, displaying response patterns that are
qualitatively similar to responses in the core. These two issues suggest that the selective regulation of the core by the BLA likely reflects the behavioral specificity required during the task.

As noted in the discussion within chapter 3, the behavioral contributions of the core and shell to this task are likely different. It follows then that the neural correlates of this behavior would likely be found within the core, not the shell. Specifically, we demonstrate that NAc core excitations in response to discrete reward-predictive cues may guide instrumental responding. The shell on the other hand, may be more involved in processing of contextual environmental stimuli (Chaudhri et al., ; Fuchs et al., 2008), in order to suppress competing behavioral responses (Ambroggi et al., 2009; Blaiss and Janak, 2009). Thus, it is still possible that the BLA regulates NAc shell neural activity, but a more specific behavioral paradigm designed to assess processing of contextual stimuli may be necessary to demonstrate this possibility.

**NAc processing of reward: limbic-motor integration**

The results presented within this dissertation provide further support for the idea that the NAc acts a site of limbic-motor integration (Mogenson, 1987). Moreover, we specifically demonstrate that limbic input from the BLA is capable of regulating several aspects of NAc signaling. A recent study has further emphasized the role of NAc integration of information (Roesch et al., 2009). In this study, a cued-instrumental task was employed, wherein cues predicted access to either low value rewards or high value rewards and directed the animal to the appropriate receptacle. The location of the more valued reward was shifted across blocks within the session, and that animals’ behavior was guided by the expected
reward and actions necessary to acquire it. NAc neural activity was recorded and the results demonstrated that whereas dopamine neurons in the VTA signal the best available option when cues are presented in this task (Roesch et al., 2007), NAc neurons reflect both the value of the action that is chosen, and the specific direction itself (Roesch et al., 2009). Our results complement these findings, demonstrating that BLA input mediates the magnitude of cue-evoked signals within the NAc. As the BLA is thought to signal value of conditioned stimuli, integration with other motivational signals within the NAc would guide the subsequent instrumental response (Cardinal et al., 2002).

This interpretation has potential implications for compulsive behavioral disorders such as drug addiction, which is characterized by robust control of motivated drug-seeking by drug-associated cues. In humans, these cues are capable of eliciting significant drug craving and feelings of negative affect (Gawin, 1991; O'Brien et al., 1992; O'Brien et al., 1998; Volkow et al., 2006). Results from chapter 4 of this dissertation demonstrate that the BLA mediates cue-evoked responses in NAc neurons in animals trained to self-administer cocaine. This finding, in conjunction with recent studies of BLA activity, demonstrates a significant neural correlate of cue-evoked drug-seeking. A recent study demonstrated that cocaine exposure induces deficits in decision-making in a reversal learning task, characterized by difficulty learning to stop responding to previously rewarded cues (Stalnaker et al., 2007). Since these deficits are mediated by persistent encoding of cues that are no longer predictive within BLA neurons, it is likely that persistent aberrant signals within the BLA in response to drug-paired stimuli drive NAc neural responses, in turn facilitating drug-seeking behaviors. Numerous behavioral studies support this mechanism as
BLA activity is necessary for drug-paired stimuli to reinstate drug-seeking behavior after extinction or abstinence (Fuchs and See, 2002; Yun and Fields, 2003; Fuchs et al., 2006).

**Future directions**

The experiments described in the preceding chapters comprise initial experiments designed to investigate the role of the NAc and NAc dopamine release in processing information related to reward-predictive cues. However, the results leave many questions to be answered, and also generate new questions that provide the basis for future research. Here, a brief discussion of future experiments that will further clarify the role of NAc signaling in associative reward processing is provided.

*Role of phasic NAc dopamine in BLA specific plasticity*

As noted above, the results in chapter 2 provide the first characterization of terminal regulation of NAc phasic dopamine that is temporally specific to presentation of cue-evoked stimuli. This suggests that BLA activity at cue-onset facilitates NAc dopamine release, in a functionally relevant manner. Previous studies indicate that MSNs undergo dopamine and NMDA dependent forms of synaptic plasticity (Calabresi et al., 2007; Pawlak and Kerr, 2008; Shen et al., 2008) and that activation of these receptors is critical for reward learning (Kelley et al., 1997; Di Ciano et al., 2001). Additionally, dopamine is important for mediating the bistable membrane potential of MSNs (i.e. up-state or down-state) (Wilson and Kawaguchi, 1996), and evidence suggests that dopamine receptor activation can have differential effects depending on which potential the MSN is currently rests (Meredith et al., 2008). Thus, it may be interesting to determine whether this transient BLA facilitation of
phasic dopamine release is related to long-term synaptic plasticity at glutamatergic synapses, or rather facilitates a transition in membrane potential which may amplify or dampen subsequent input. Future studies could employ in vivo intracellular recording of NAc neurons, coupled to stimulation of the BLA to determine the specific changes that occur at the post-synaptic membrane potential.

*Application of optogenetic techniques to assay NAc signaling*

The results of this dissertation have attempted to establish functional relationships between NAc signaling, BLA activity and associative reward processing. Here, a relatively non-selective technique was employed (micro-infusion of GABA agonists) to inhibit the BLA. While these results provide extremely useful information, particularly coupled to measurement of downstream signaling, that functionally characterize previously undescribed neural connectivity, this technique lacks specificity and temporal precision to sample specific synaptic connections and cell populations that may govern a given behavior. Recent advances in the emerging field of optogenetics provide a novel tool to assay the afferent modulation of NAc signaling, and further isolate the specific cell populations involved in guiding reward processing.

With optogenetics, optical excitation or inhibition of neurons can be accomplished through the introduction of light-sensitive channels, such as the algae protein, Channelrhodopsin-2, through genetic techniques (e.g. viral transfection) to neural tissue (Boyden et al., 2005). These channels can be targeted to specific neuronal subtypes through the use of cell-specific promoters (Adamantidis et al., 2007) or cell-specific recombination in
populations that express CRE recombinase (Cardin et al., ; Tsai et al., 2009), and subsequently manipulated through applying optical stimulation to these populations.

For example, the results of this dissertation argue strongly that ongoing signaling within the NAc is significantly altered through manipulation of afferent neural substrates such as the BLA. However, inactivation of the BLA using GABA agonists does not provide a complete demonstration of the mono-synaptic regulation of NAc activity. Optogenetic techniques could provide this answer through infection of BLA projection neurons with optogenetic channels, which in turn are transferred to the terminal regions of these neurons. Optical probes could then be placed within the NAc (or any BLA termination for that matter) and then stimulated, or inhibited depending on the optogenetic channel employed, to isolate the direct influence of projections from the BLA.

As noted previously, the NAc receives convergent input from a number of brain nuclei that have been implicated in many different aspects of reward (Brog et al., 1993; Zahm, 1999). Thus, it can be assumed, that through circuit-level manipulation of these neural substrates, the functional contribution of NAc signals can be more precisely understood. For example, recent evidence has demonstrated that inactivation of the dorsomedial PFC attenuates cue-evoked signals within the NAc (Ishikawa et al., 2008b). This attenuation is accompanied by a different behavioral change from BLA inactivation (Ishikawa et al., 2008a), as dmPFC inactivation produces an increase in responding for the non-associated cue (Ishikawa et al., 2008a, b). Optogenetic control of these afferent inputs would greatly advance our understanding of the neural circuitry involved in these processes.

Much of this dissertation has emphasized the afferent modulation of NAc signaling, based primarily on the phasic response patterns that these neurons exhibit during behavior.
However, a significant question that remains unanswered concerns the characteristics of these NAc neurons themselves. As noted in the opening chapter, MSNs within the striatum are largely differentiated based upon their neurochemical composition (i.e. enkephalin or dynorphin) (Voorn et al., 1989) and expression of dopamine receptors (Le Moine and Bloch, 1995). To date, no study has been able to simultaneously record the neurophysiological activity of NAc neurons and also identify their neurochemical profile. Again, advances in genetic engineering in rodent models, and optical control of neural activity provide a unique opportunity to answer this question. Specifically, genetically engineered mice that express green fluorescent protein (GFP) tagged dopamine receptors could be virally transfected with optical channels, such that only D1 containing neurons express the optical channel. A dual-probe consisting of a microelectrode and optical stimulating device could be placed within the NAc to record the activity of neurons while an animal performs a task (Cardin et al., 2009; Zhang et al., 2009), and also stimulate the cell-specific population to identify which type of MSN is displaying a given response pattern.

Effects of chronic cocaine on associative processing

Experimental evidence demonstrates that drug addiction is associated with impaired decision-making processes. For example, human addicts often exhibit impulsive choice behaviors, discounting future rewards much sooner than non-addicts, attributing an augmented value assessment of immediate rewards (Bickel et al., 1999; Kirby et al., 1999). Animal models have demonstrated a similar pattern of behavioral dysfunction (Woolverton et al., 2007). Additionally, numerous neuroadaptations within the NAc are associated with chronic cocaine exposure (Wolf et al., 2004; Hyman et al., 2006), reflected in altered NAc
signaling (Hollander and Carelli, 2007). Future studies could determine what changes cocaine or other drugs of abuse induce within NAc signaling, or within the distributed afferent neural substrates. Specifically, rats could be trained on a cued-instrumental task for sucrose, where cues predict either immediate, small rewards, or delayed, large rewards. After training, rats could be exposed to cocaine, either contingently or non-contingently (different groups). Following this exposure, either NAc dopamine release or neural activity could be examined to investigate whether cocaine disrupts signaling. These designs could also examine whether afferent control of NAc signaling is shifted in cocaine-experienced animals (e.g. decreased PFC control or disinhibition of inappropriate reward-seeking). The results of these studies would greatly advance our understanding of processes that govern compulsive drug-seeking behavior, and provide potential sites for therapeutic treatment of such disorders.

Concluding remarks

Rewards are not procured and consumed in a vacuum. Therefore, the ability to pursue, procure and consume rewards such as food, water, sex and social interactions sets the foundation for successful adaptive responding. This ability is mediated by a diverse network of brain nuclei, including the NAc and its afferent connections from the BLA (see Figure 1.1, Chapter 1). The experiments described in this dissertation reveal that functional regulation of the neurochemical and neurophysiological signals within this network guide appropriate processing of reward-predictive cues. Taken together, these experiments provide vital insight into how this network operates while an organism actively engages in the pursuit of reinforcement, specifically during cued-instrumental responding. However, this dissertation
merely expands on the expansive literature that demonstrates that the NAc is a critical component to appropriate behavioral output, as dysfunction within the region has been linked to numerous psychiatric conditions such as drug addiction, gambling, obesity, attention disorders, schizophrenia and depression (Carlsson, 1978; Volkow and Wise, 2005; Nestler and Carlezon, 2006; Tamminga and Nestler, 2006; Scheres et al., 2007). Understanding how environmental stimuli come to regulate, or as is often the case in these disease states, aberrantly control goal-directed behaviors is critical to developing future treatments and effective therapeutic methods for these complex behavioral disorders.
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